

## EXHIBIT

A1

FRO

SITY MMI

FAX NO. : 3147733403

Dec. 19 2002 10:21AM P2

Counts			871972 plaque assays
			(Cumulative)
<del>1041</del>	<del>19</del> 106	88	<del>37</del> 120
	<del>20</del> 83	108	<del>38</del> 114
	<del>21</del> 84	95	<del>39</del> 123
	<del>22</del> 13	14	40 16
	<del>23</del> 12	14	41 12
	<del>24</del> 5	12	42 12
<del>1035</del>	<del>25</del> 7	omit → cells dead	<del>43</del> 1
	<del>26</del> 69	78	44 1
	<del>27</del> 68	74	45 1
	<del>28</del> 11	14	46 0
	<del>29</del> 9	8	47 1
	<del>30</del> 9	10	48 0
<del>1038</del>	<del>31</del> 110	41	<del>49</del> 46
	<del>32</del> 107	37	50 57
	<del>33</del> 85 (but high cell death)	41	51 52
	<del>34</del> 16	5	52 - (total cell death)
	<del>35</del> 7	1	53 6
	<del>36</del> 9	6	54 8

Ad 5 → distinct plqs  
 1035 → not as definitive  
 1038 →

1041 → more distinct plq  
 918 → distinct  
 305 → distinct

large plaques  
 716 → largest, most distinct plaques

small plaques  
 801 → <sup>very</sup> small + slow growth  
 712 → very small with slow growth

A2

11.6K → possibly related to plaque size  
→ morphology?

(tend to vary more  
in plaque size.)

### Plaque Size

#### Larger & distinct

dl 753 (exp.)

dl 754

dl 714

dl 778

Ad 5 (exp.)

dl 799

~~dl 718~~

dl 716 (exp.)

dl 751 (exp.)

dl 718

#### Smaller (less distinct)

most SPY 10.4K mutant

[pm 775 more distinct &amp; larger]

pm 771

dl 713

dl 327 (smaller than  
rec 700 in dl 728)

#### Small

748

712

dl 801

dl 766

dl 742

pm 785

In 724

dl 748

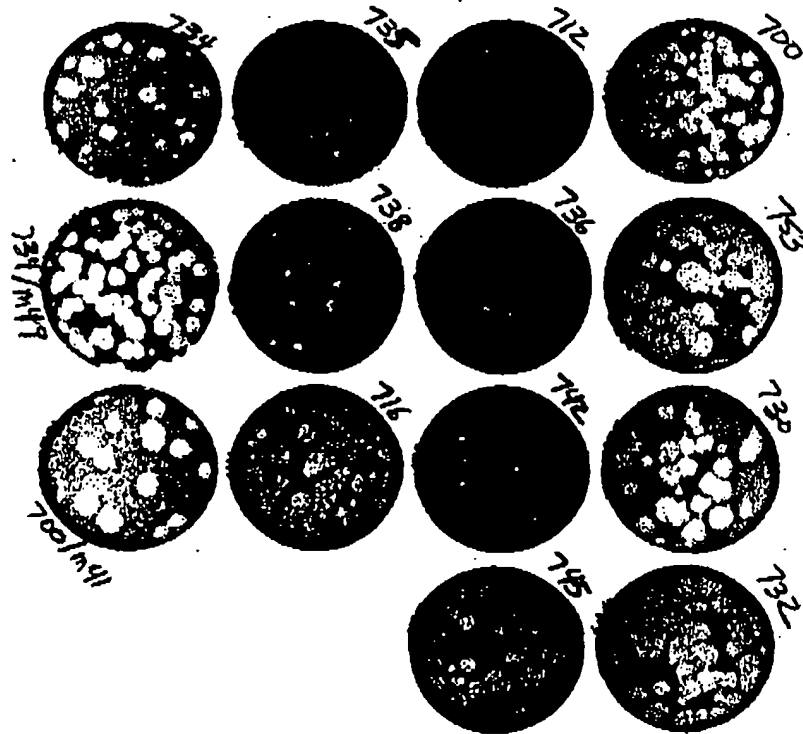
dl 706

dl 708

dl 717

dl 7001

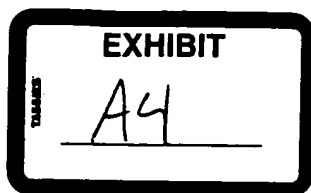
## E3-11.6k Small Plaque Mutants



Early work  
on defining  
ADP function

EXHIBIT

A3



Plaque Assay (11.6K mutants <sup>to test</sup> ~~plaque assay~~)

Uima	Prep	Titer	5*10 <sup>-7</sup>	5*10 <sup>-10</sup>
① rec 700 (VS188) (910513)		2.37*10 <sup>11</sup>	2	3
② dl 742 (860313)		1.77*10 <sup>11</sup>	2	3
③ dl 745 (860313)		2.06*10 <sup>11</sup>	2	3
④ dl 712 (ply 1) (VS204) (911101)		1.56*10 <sup>11</sup>	2	3
⑤ dl 730 (39-4) (VS168) (901214)		1.93*10 <sup>11</sup>	2	3
⑥ dl 732 (29-1) (AS) (890414)		3.4*10 <sup>11</sup>	2	3
⑦ pm 734 (19-2) (890504)		6.7*10 <sup>10</sup>	3	2
⑧ dl 735 (34-1) (890425)		9.*10 <sup>10</sup>	3	2
⑨ dl 736 (0.04-1) (890414)		2.88*10 <sup>10</sup>	3	2
⑩ dl 738 (38-11) (VS171) (910118)		?	3	3
⑪ 700/m41 (AS) (920214)		?	3	3
⑫ pm 734/m49 (AS) (920402)		?	3	3
⑬ dl 716 (ply. 4) (VS125) (900202)		2.04*10 <sup>11</sup>	1	2
⑭ dl 753 (VS78) (880624)		1.92*10 <sup>11</sup>	1	2
⑮ mock		-	(150)	

SF-DME for all pre-infection;  
infection with 0.5ml of dilutions at 4:30pm;  
added 6ml of overlay at 5:45pm;

(5/24) added 5ml of NR overlay; one dish of  
dl 738 (5\*10<sup>-7</sup> dilution) dropped → discarded

on (5/24) see large distinct plaques on dl 730, dl 732,  
dl 716, dl 753 dishes (+ some dl 115), rec 700 may  
have a few smaller plaques & really do not  
see plaques on other dishes yet.



EXHIBIT

A5

(7/1) plagues marked in red  
(6/3) marked in green

## Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
① rec 700 (V5188)(910503)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+39 55 +8 2	+35 51 +4 2	- +4 3	quite large + distinct vary in size how distinct (smaller & less distinct) (than 730, 732, 753)
② dl 742 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+23 0 0	+52 +1 0	- 0 0	very indistinct very tiny + indistinct
③ dl 745 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+20 24 +2 5	+13 22 +1 1	- +2 3	quite large to <sup>or</sup> slightly quite distinct Similar size to rec 700
④ dl 712 (ply 1) (V5204)(911101)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+8 0 +1 0	+6 0 0 0	- 0 0	no plga visible (at least not definite) very small plagues
⑤ dl 730 (39-4) (V5168)(701214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+16 20 +1 1	+22 20 +4 3	- +1 7	quite large to distinct larger than rec 700
⑥ dl 732 (29/1) (A5)(890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+8 72 +3 4	+12 52 +3 1	- +3 5	larger & more distinct than dl 730 & dl 753 large & very distinct
⑦ pm 734 (1/2) (890504)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+16 4 +4 0	+8 1 +2 0	+20 5 -	probably not very small, but very indistinct than rec 700
⑧ dl 735 (39/1) (890425)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+8 6 0 0	+1 0 +1 0	+13 - -	indistinct plagues, size not very definite very small + indistinct
⑨ dl 736 (0.0/1) (890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+1 1 0 0	+2 1 0 0	+4 1 -	indistinct plagues very small + indistinct

EXHIBIT

A6

(731) plaques

(93) marked in green

## Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (V5171)(710118)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	$\times 6$ 4 0 0	$\times 1$ 11 0 0	— 0 0	not too small, but not very distinct small & indistinct
(11) 700/m41 (ASX920214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	$\times 7$ 8 $\times 0$ 2	$\times 3$ 11 $\times 2$ 0	$\times 12$ 10 $\times 1$ 2	~ like rec 700 quite large & very distinct
(12) pm 734/m41 (AS) (920402)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	$\times 55$ 28 $\times 11$ 3	$\times 13$ 31 $\times 6$ 4	$\times 46$ 36 $\times 7$ 1	~ like rec 700 ~ like rec 700
(13) dl 716 (ply 4) (V5125)(700202)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	$\times 26$ 62 $\times 1$ 5	— $\times 1$ 3	— —	quite large & distinct larger & more distinct than rec 700
(14) dl 753 (V578)(880624)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	$\times 15$ 42 $\times 2$ 2	— $\times 0$ 3	— —	quite large & distinct very large & distinct
(15) mock	—	0 0	—	—	good monolayer

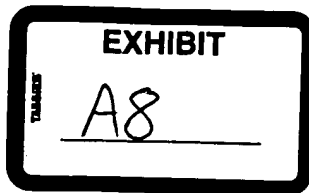
EXHIBIT

A7

Counts of (F<sub>25</sub>) Plaque Assays

Sample	Dilution	A	B	C	Comments
① rec 700 (V5188)(910503)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+13 +0	+16 +3	- +1	plaques quite larger & distinct, new plaques distinct
② dl 742 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+6 0	+5 +1	- 0	new plaques very indistinct, older plaques still small with diffuse edges
③ dl 745 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+7 +2	+7 +0	- +1	large, distinct plaques; new plaques very distinct
④ dl 712 (plg 1) (V5204)(911101)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+6 +0	+10 0	- 0	small & indistinct older plqs still small
⑤ dl 730 (39-4) (V5168)(901214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+6 +1	+8 +1	- +2	very large & very distinct plaque new plqs distinct
⑥ dl 732 (29) (A5)(890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+4 +0	+2 +4	- +1	very large & distinct plaque
⑦ pm 734 (19/2) (890504)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+19 +2	+17 +1	+13 -	plaques a bit smaller than rec 700; new plaque quite distinct
⑧ dl 735 (34/1) (890425)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+6 +1	+12 +1	+13 -	small & very indistinct plaq
⑨ dl 736 (0.04/1) (890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+5 +1	+5 0	+6 -	very small & very indistinct (both old & new)

(4/5) mar 11/04 m - P



# Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (V5171)(910118)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+5 +1	+6 0	- 0	new plaques very small & indistinct, even older plaques are ~ 0 size with diffuse edges
(11) 700/m41 (AS)(920214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+5 +0	+2 +0	+5 +0	plaques large, new plaques very distinct (similar size to much older 10728)
(12) pm 734/m49(AS) (920402)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+17 +4	+16 +1	+17 +5	new plaques very distinct & fairly large & distinct, maybe a bit smaller than rec 700 plaques
(13) dl 716 (plg 4) (V5125)(900202)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+1 +1	- +1	- -	very large & distinct (exp. on $10^{-10}$ )
(14) dl 753 (V578)(880624)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+7 +1	- +2	- -	very large & distinct
(15) mock	-	0	-	-	good monolayer

EXHIBIT

A9

## Counts of (5/25) Plaque Assays

Sample	Dilution	A	B	C	Comments
① rec 700 (V5138)(910503)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+3 +1	+6 +1	- +1	distinct fairly large plaques
② dl 742 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+20 +3	+22 +2	- +1	small + indistin. plaques; new plaque "pinpoint"
③ dl 745 (860313)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+7 +0	+2 +0	- +1	large distinct plaques
④ dl 712 (ply 1) (V5204)(911101)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+28 +1	+29 +1	- +2	very small plga; new plga "pinpoint"
⑤ dl 730 (39-4) (V5168)(901214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+1 +1 (mol'd)	+4 +0	- +0	very large, nice plaques (edges a bit diffused)
⑥ dl 732 (29) (A5)(890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+7 +0	+1 +0 (mol'd)	- +1	very large, nice plaques
⑦ pm 734 (1 1/2) (890504)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+28 +2	+28 +3	+25 -	slightly small plaques; new plaques fairly big + distinct
⑧ dl 735 (3 1/4) (890425)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+18 +5	+34 +7	+30 -	older plga + new new plga + "pinpoints"
⑨ dl 736 (0.04) (890414)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+11 +0	+22 +0	+15 -	very small plga new plga "pinpoints"

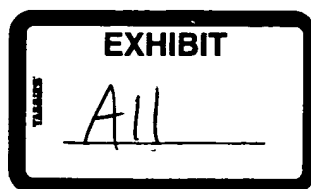
EXHIBIT

A10

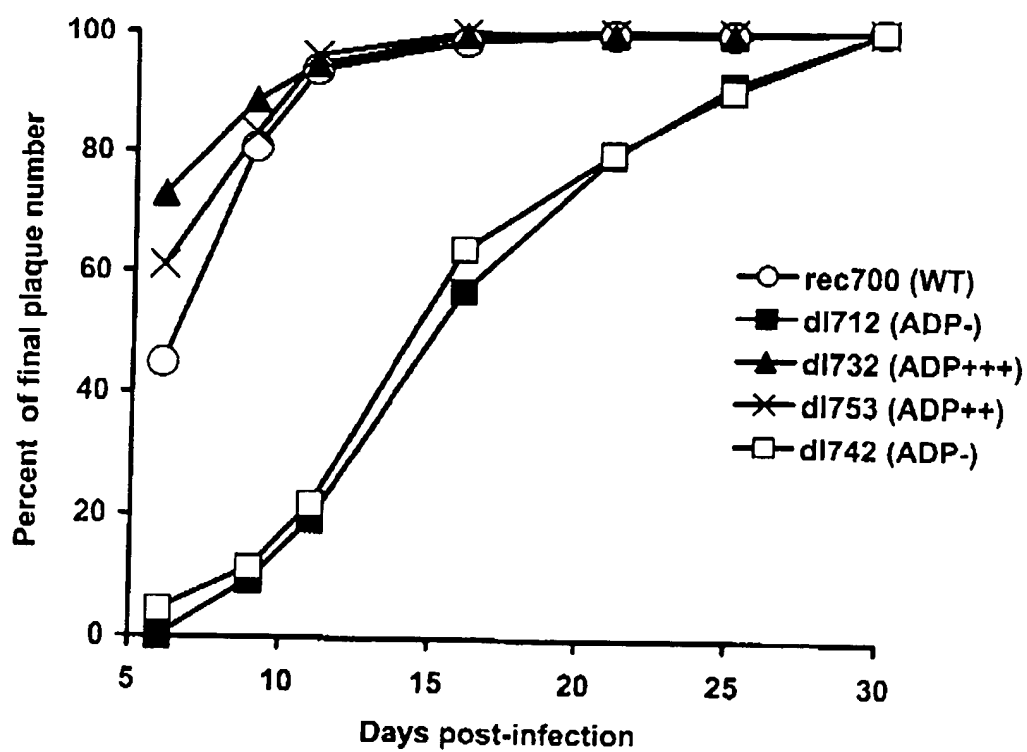
(178) marked in black.

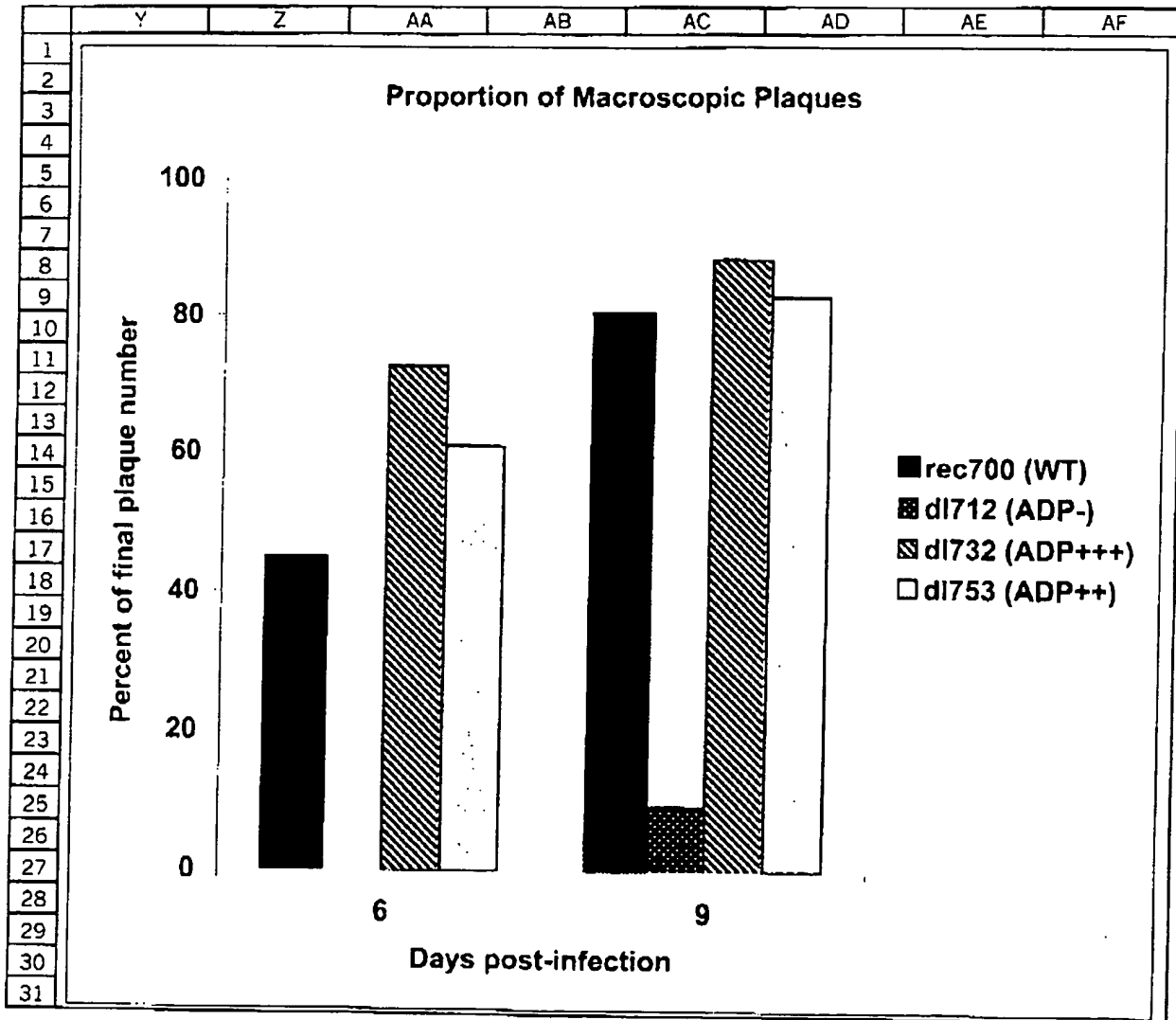
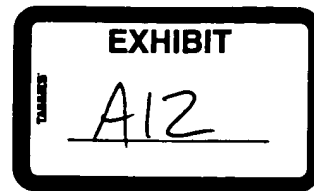
## Counts of (5/25) plaque Assays.

Sample	Dilution	A	B	C	Comments
(10) dl 738 (38-11) (VS171)(910118)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+22 +2	+16 +3	- +3	small + indistinct, new plaques very small
(11) 700/m41 (AS)(920214)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+3 +1	+5 +0	+4 mm +0	new plaques larger than oldest of dl 738; large plaques + distinct
(12) pm 734/m49(AS) (920402)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+5 +2	+7 +3	+15 mm +4	large distinct plaques (new plaques larger than oldest (738))
(13) dl 716 (ply 4) (VS125)(900202)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+3 +1	- +1	- -	very large + distinct
(14) dl 753 (VS 78)(880624)	$.5 \times 10^{-9}$ $.5 \times 10^{-10}$	+1 +0	- +2	- -	very large + distinct
(15) mock	-	0	-	-	good monolayer



plaque assay



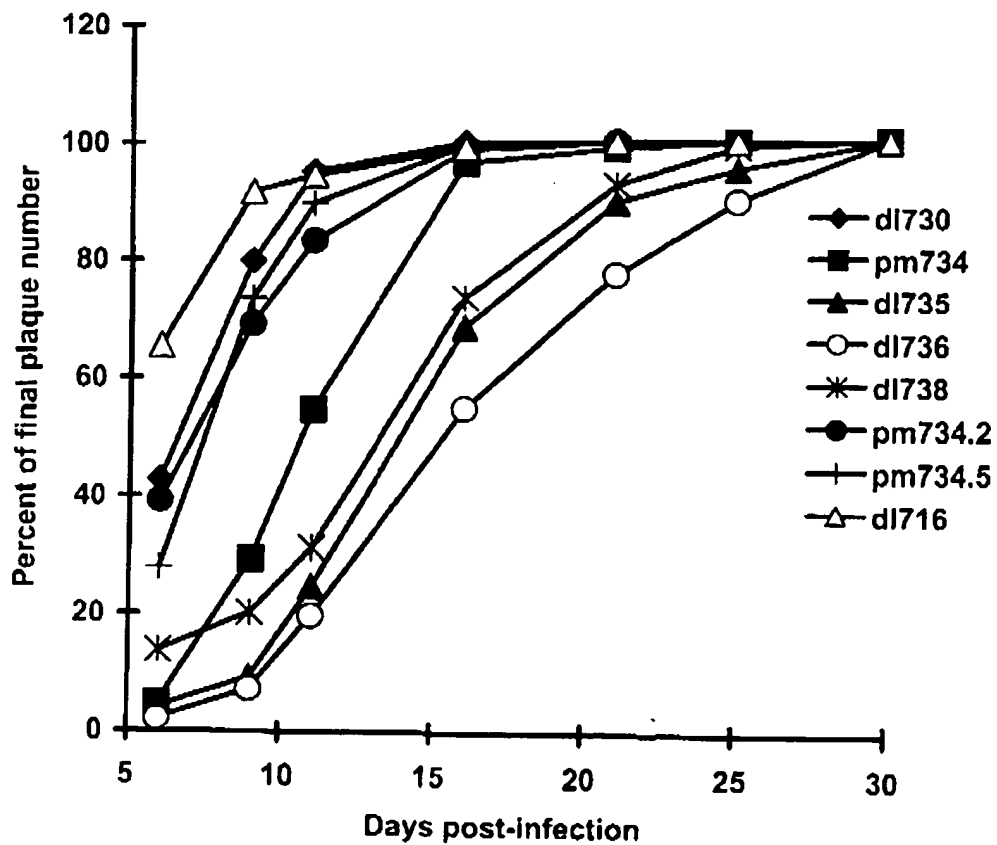




**EXHIBIT**

A13

## plaque assay

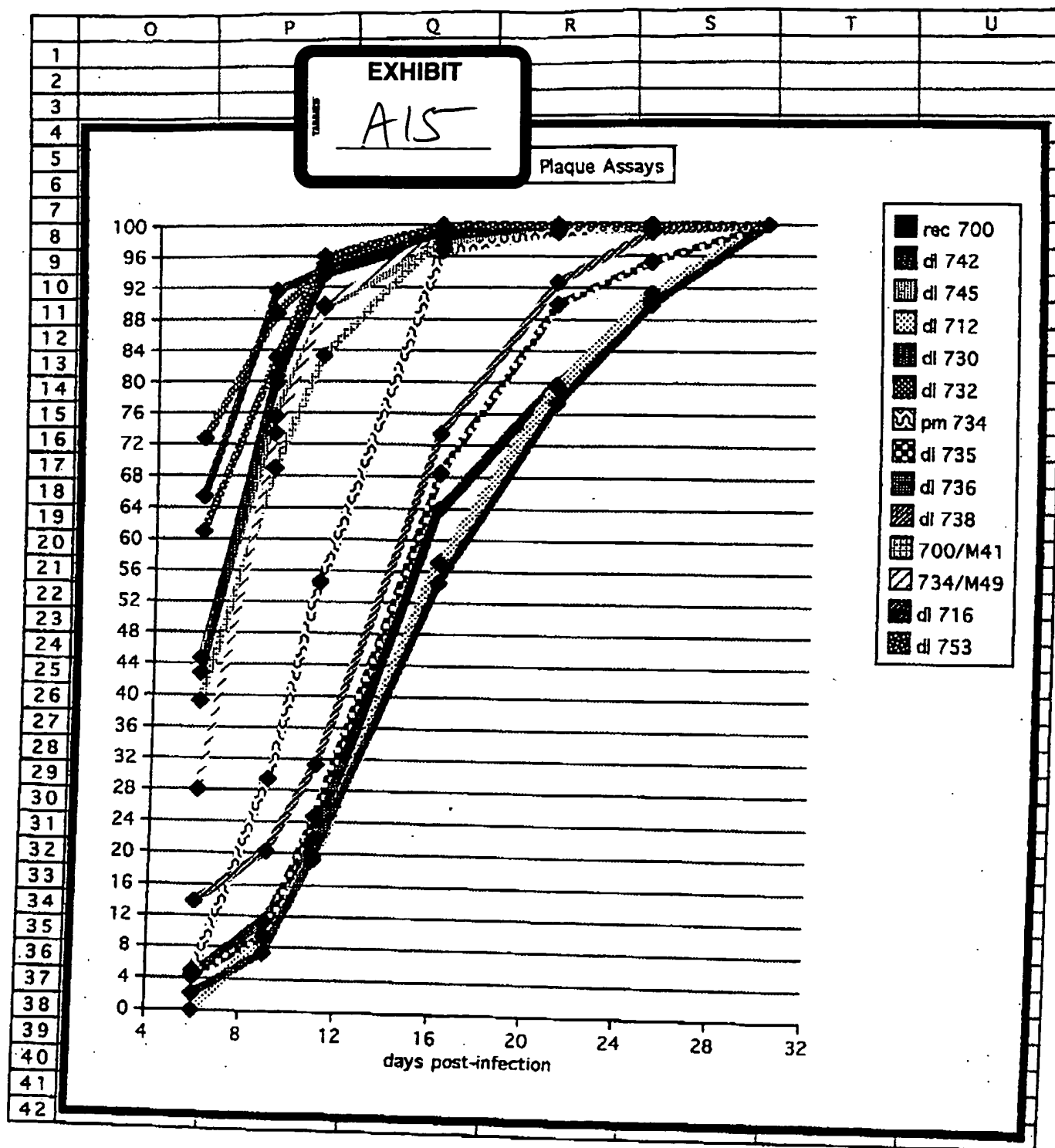


## EXHIBIT

TABLE

A14

		B	C	D	E	F	G	H
2		6	9	11	16	21	25	30
3	rec700 (WT)	44.8	80.6	93.7	98.4	99.6	99.6	100
4	dl712 (ADP-)	0	9.3	19.3	57.1	79.5	91.3	100
5	dl732 (ADP+++)	72.8	88.6	94.6	99.5	99.5	99.5	100
6	dl753 (ADP++)	61	83.1	96.1	100	100	100	100
7	dl742	4.4	11.4	21.9	64	79.8	90.4	100
8	dl745	44.7	75.6	89.4	97.6	99.2	99.2	100
9		6	9	11	16	21	25	30
10	dl730	42.9	79.8	95.1	100	100	100	100
11	pm734	4.9	29.3	54.6	96.6	99	100	100
12	dl735	4.2	9.3	24.7	68.4	89.8	95.3	100
13	dl736	2.2	7.3	19.7	54.7	77.4	89.8	100
14	dl738	13.8	20.2	31.2	73.4	92.7	99.1	100
15	pm734.2	39.3	69	83.3	98.8	100	100	100
16	pm734.5	27.9	73.4	89.7	99.5	100	100	100
17	dl716	65.4	91.6	94.4	99.1	100	100	100
18								
19								
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31								



EXHIBIT

116

p. : 3147733403

**SECRET**

[illegible]

**EXHIBIT**  
**TAB**  
A17

**EXHIBIT**  
**TAB**  
A17

FROM :SAINT LOUIS UNIVERSITY MMI

FAX NO. : 314

19 2002 10:30AM P16

P.A. (p. 2)

[illegible]

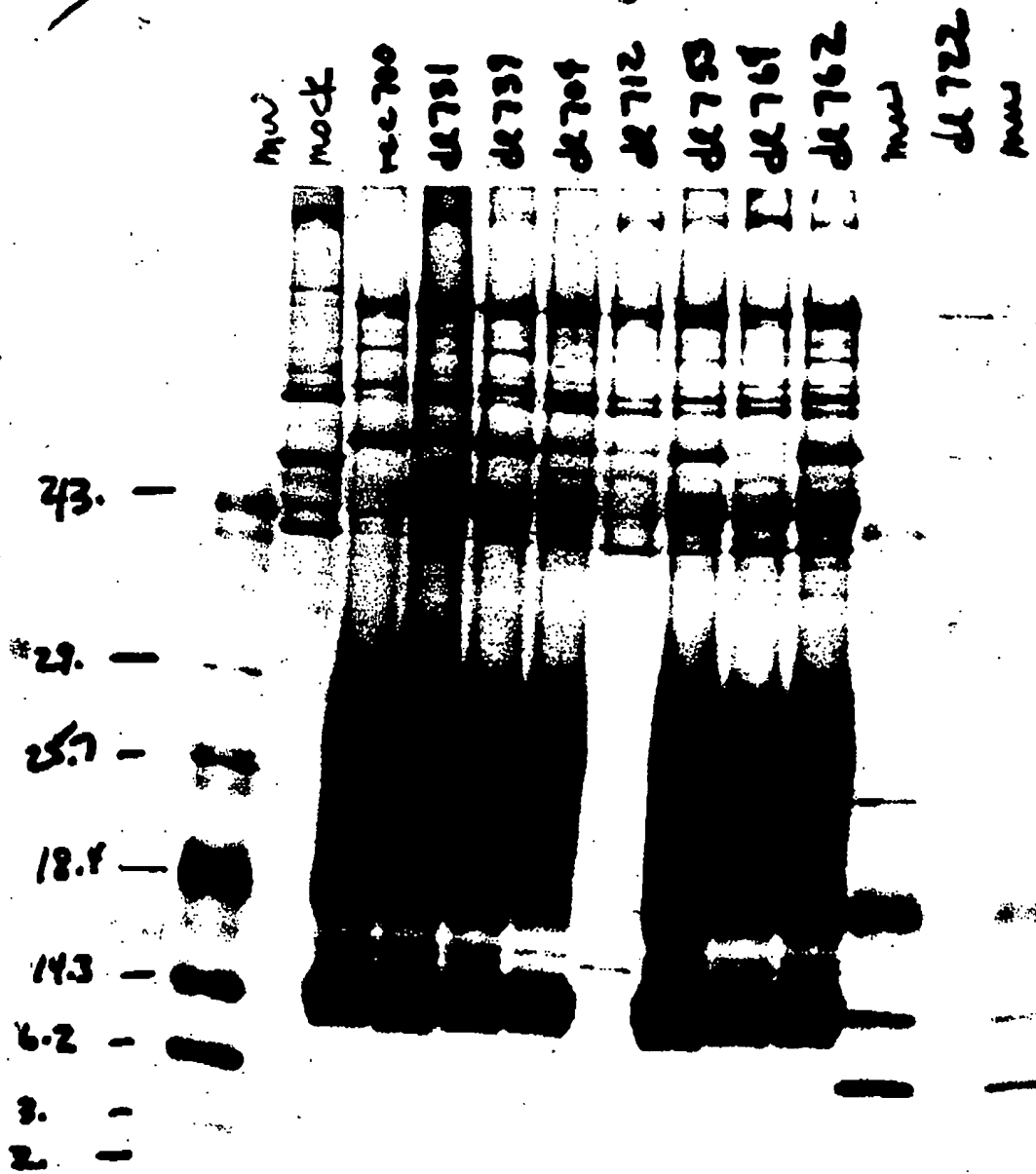


EXHIBIT  
A18

Gel #1

13 day exposure  
5 min developer

709 #1

11.6 (Deletion Mutation)

8/9 →

43. —

29. —

25.7 —

18.1 —

14.3 —

6.2 —

3. —

2. —

EXHIBIT

A19

Gal #1

13 day exposure

5 min developer

709 #1

11.6 (Petition Act)

8/9 →

EXHIBIT

A20

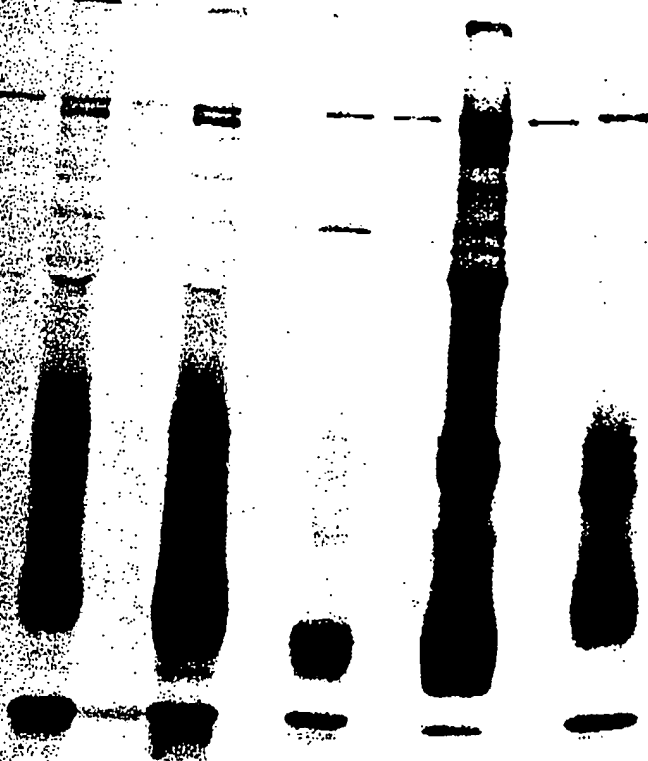
FROM : SAINT LOUIS UNIV

:3147733403

Dec. 19 2002 10:39AM P4

7 day exposure (5 min developer)

9cc 700  
Ab 730  
Ab 745  
Ab 732  
Ab 742  
Ab 735  
Ab 736  
Ab 737  
Ab 738  
Ad2





EXHIBIT

A21

Stained by A.S.

set #1		set #2		set #3	
1	mock $\alpha 11.6(2-16)$	13	714 $\alpha 11.6(2-16)$	25	732 $\alpha 11.6(87-101)$
2	rec 700 $\alpha$ Fiber	14	715 $\alpha$ Fiber	26	742
3	dl 712	15	713	27	735
4	730	16	25-mer	28	737
5	745	17	734	29	738
6	732	18	734/MS6	30	35-mer
7	742	19	734/M49	31	714
8	735	20	mock $\alpha 11.6(87-101)$	32	715
9	736	21	rec 700	33	25-mer
10	737	22	dl 712	34	734
11	738	23	730	35	734/56
12	35-mer	24	745	36	734/49

1st Ab's:

 $\alpha 11.6(2-16)$  (910830 preads 2X; 1:4 dil)

#98689 bleed out

$$800 \mu\text{l} \left[ \begin{array}{l} (2-16) (1:200) \leftarrow 16 \mu\text{l} \\ \text{Fiber} (1:500) \leftarrow 1.6 \mu\text{l} \leftarrow \text{mt} \end{array} \right.$$
 $\alpha 11.6(87-101)$  (910830 preads 4X; 1:4 dil)

#98686 bleed out

$$720 \mu\text{l} \left[ (87-101) (1:400) \right. \quad \frac{7.2 \mu\text{l}}{712.8}$$

2nd Ab's:

(all 1:50 dil's)

[  $\alpha$  Rabbit - FITC /  $\alpha$  Mouse - FITC ]or  $\alpha$  Rabbit - FITC

A22

slides)

## 920730 Descriptions of AS 11.6K Mutants (FF)

① - ~~①~~  $\alpha$  11.6K (Z-16) (1:200 dil) (1:400  $\alpha$  Fiber-RITC)① L-mock - ~ no backgd for Fiber; slight background for  $\alpha$  11.6K (esp. for mitotic spindle poles)

R-rec 700 - all in late stage (by Fiber staining); ~20-30% of cells stain for 11.6K (n.e. &amp; Golgi primarily), some "debris" or aggregated material

② L-dl 712 - 100% inf'n; no 11.6K staining

R-dl 730 - 20-30% stain for 11.6K  $\rightarrow$  maybe more Golgi & debris staining; 100% infected③ L-dl 745 - ~100% inf'n; poss. very diffuse Golgi staining  $\rightarrow$  not very bright & see no n.e. stainingR-dl 732 - 70-80% stain for 11.6K  $\rightarrow$  n.e., Golgi (Golgi staining reduced); a lot of aggregated material

④ L-dl 742 - looks ~ like dl 712

R-dl 735 - 100% inf'n; very little 11.6K staining (poss a bit in Golgi, not really n.e.)

⑤ L-dl 736 - 100% inf'n; little if any staining; poss some aggreg'd material but not definitive

R-dl 737 - 100% inf'n; bright staining; 30-40% of cells stained  $\rightarrow$  n.e., debris & virus replication sites

⑥ L-dl 738 - 100% inf'n; 11.6K aggregated material

R-35-mex - " " ; not so much n.e. staining &amp; Golgi staining is very diffuse; more e.r.?

⑦ L-dl 714 - ~30% of cells stained, good n.e. &amp; Golgi but also a lot of e.r. (do not see plasma membrane)

R-dl 715 - like 714 except less n.e. staining &amp; more aggregated material

⑧ L-pm 734 - like 712

R-734/mSL - ben. on

" " " " " " " "

EXHIBIT

A23

## Descriptions (cont'd)

- ⑨ L-dl 713- 50-60% stained,  $\alpha$  n.e., Golgi + a lot of e.r. - maybe more aggregated material  
R- 25-mer - less n.e. staining; seemingly more Golgi staining; seemingly less staining overall
- ⑩ L-pm 734/m49 - like dl 712  
#11-20  $\alpha$  11.6K (87-101) (1:400 dil.)
- ⑪ L-mock - slight Golgi-like pattern (very light)  
R-rec 700 - ~100% stained, good n.e., Golgi; more e.r. + particulate than sometimes
- ⑫ L-dl 712 - a bit anti-nuclear (not 11.6K pattern)  
R-dl 730 - ~100% of cells; very bright, maybe a bit more into all membranes (but n.e. + Golgi most)
- ⑬ L-dl 745 - more "grainy" staining + more aggregated material, otherwise quite "normal"  
R-dl 732 - almost 100% with very bright n.e. staining, also Golgi + some e.r. (brighter than rec 700)
- ⑭ L-dl 742 - like 712  
R-dl 735 - almost only Golgi (very reduced n.e.)
- ⑮ R-dl 737 - very bright; typical pattern but more particulate + more e.r.
- ⑯ L-dl 738 - not in membranes; some diffuse staining but mainly lots of aggregated material  
R-35-mer - bright; in all membranes
- ⑰ L-dl 714 - very bright; in all membranes  
R-dl 715 - less n.e. and more in plasma membrane (than dl 714)
- ⑱ R-25-mer - more Golgi, less n.e., vesicles or particles in cytoplasm
- ⑲ L-pm 734 - fairly typical but "grainier" appearance  
R-734/ms6 - sim to p 734 but not quite as bright
- ⑳ L-734/m49  $\rightarrow$  sim to 734, maybe more Golgi +
- carriers out. etc.

43 —

29 —

184 —

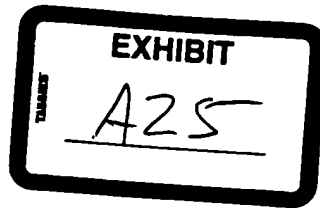
143 —

62 —

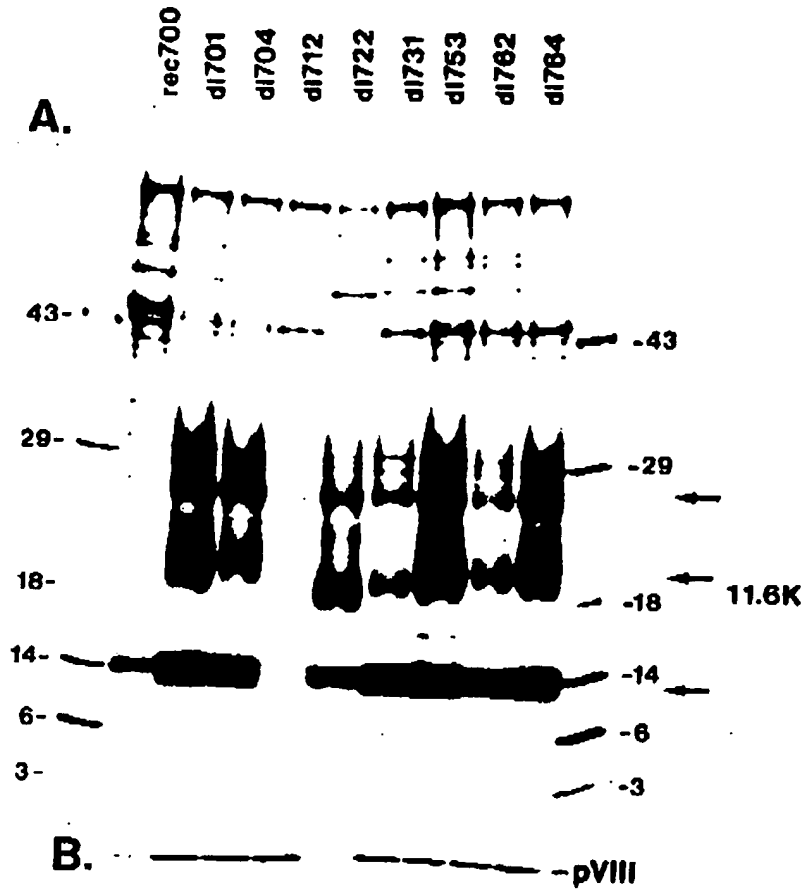
EXHIBIT

A24

#1 100% 4 day exposure



#  
IF  
910827  
pub. #?



Have all these  
prints.

Fig. 1

900331 gel #1  
~ 11.6K (87-101)  
like ext. v. d. v.

(43) #1 ← (41/2) IF 1/2 PELL

A26

(checking for 11.6.1-70)

## Assays of Virus Release from A549 cells

Sample #	Infection	Sample Plated cell #	pfu	Time (p.i.)	PA date	PA dilution (all 5#)
①	mock	$2 \times 10^6$	<del>10<sup>5</sup></del>	7 1/2 h	<del>6/28</del>	-
②	mock	$5 \times 10^5$	-		-	-
③	rec 700	$2 \times 10^6$	$10^5$		(6/28) AT	$5 \times 10^0, 10^{-1}$
④	↓	$5 \times 10^5$	$10^5$			$10^0, 10^{-1}$
⑤	↓	$2 \times 10^6$	$10^3$			$10^0$
⑥	dl 712	$2 \times 10^6$	$10^5$			$10^0, 10^{-1}$
⑦	↓	$5 \times 10^5$	$10^5$			$10^0, 10^{-1}$
⑧	↓	$2 \times 10^6$	$10^3$			$10^0$
⑨	↓	$5 \times 10^5$	$10^3$			$10^0$
⑩	dl 753	$2 \times 10^6$	$10^5$			$10^0, 10^{-1}$
⑪	↓	$5 \times 10^5$	$10^5$			$10^0, 10^{-1}$
⑫	↓	$2 \times 10^6$	$10^3$			$10^0$
⑬	mock	$2 \times 10^6$	-	24 h	(6/29) AT	$10^0$
⑭	mock	$5 \times 10^5$	-			$10^0$
⑮	rec 700	$2 \times 10^6$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
⑯	↓	$5 \times 10^5$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
⑰	↓	$2 \times 10^6$	$10^3$			$10^0, 10^{-2}, 10^{-4}$
⑱	dl 712	$2 \times 10^6$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
⑲	↓	$5 \times 10^5$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
⑳	↓	$2 \times 10^6$	$10^3$			$10^0, 10^{-2}, 10^{-4}$
㉑	↓	$5 \times 10^5$	$10^3$			$10^0, 10^{-2}, 10^{-4}$
㉒	dl 753	$2 \times 10^6$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
㉓	↓	$5 \times 10^5$	$10^5$			$10^{-1}, 10^{-3}, 10^{-5}$
㉔	↓	$2 \times 10^6$	$10^3$			$10^0, 10^{-2}, 10^{-4}$

at indicated times p.i., 1 ml of sup was removed, cells were pelleted (microfuge, 3 min) & sup removed to new Eppendorf; PA's of 0.5 ml volumes (single dishes); added back 1 ml of fresh DMEM (2% FCS) to each dish to replace volume removed



SCHOOL OF MEDICINE

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Department of Molecular  
Microbiology & Immunology

William S. M. Wold, Ph.D.  
Professor and Chairman

Dr. Rae Lyn Burke  
Chiron Corporation  
Department of Virology  
4560 Horton Street  
Emeryville, CA 94608

Dear Rae Lyn,

I have finally returned to my office and cleared off the stack of work on my desk. Thank you for providing the names of venture capitalists, and for contacting Rajen Dalal. I have decided to take the less visionary approach mentioned in your letter of June 10, 1994, and to request funding from Chiron for the gene therapy project on the adenovirus E3-11.6K protein that promotes cell death. The proposal is enclosed. I have submitted a patent application for this project; if the patent is awarded, it would provide protection in the U.S.A. but not in Europe or Asia.

Also enclosed is a budget for the proposal. I anticipate carrying out the research in my laboratory here at St. Louis University. However, other alternatives, including doing the work at Chiron, are also possible.

I have requested a consultant fee for myself and Dr. Ann Tollefson. Ann is an Associate Research Professor working in my laboratory; she is the co-discoverer of the cell death-promoting properties of 11.6K. She will co-direct the project and she will perform benchwork on the project. Note that our consultant fees are separate from the requested budget for the project.

I am also considering establishing a corporation, perhaps in August, with St. Louis University as a minor partner. If Chiron decides to fund this project, perhaps the funding can be channeled through this corporation. Therefore, the budget for the project should be considered to be tentative.

Our Biotechnology Transfer Office requires that an appropriate official from Chiron sign a confidentiality agreement. The form is enclosed.

Dr. Rae Lyn Burke

page 2

Thank you again for your interest, and for your hospitality during my visit.

With best regards,

A handwritten signature in cursive script, appearing to read "Bill".

William S. M. Wold, Ph.D.  
Professor and Chairman

WSMW:jlrm

enclosures



## BUDGET

### Personnel:

Lynda K. Hawkins, Ph.D.	\$20,700
TBA Postdoctoral Fellow	25,600
Fringes	9,955
Subtotal Personnel	56,255

<u>Supplies:</u>	24,000
------------------	--------

### Other:

Maintenance contracts and repair	1,200
Telephone, postage, photocopier demurrage	400
Radiation disposal	500
Publication, illustrations	500

Subtotal other expenses	2,600
-------------------------	-------

Subtotal	82,855
----------	--------

Indirect costs (10%)	8,286
----------------------	-------

TOTAL	\$91,141
-------	----------

Consultant costs*	\$12,000
-------------------	----------

\*In addition to total project budget.

CONFIDENTIALITY AGREEMENT

THIS AGREEMENT is made by and between St. Louis University Medical Center having a principle place of business located at 1402 South Grand Blvd., St. Louis, MO 63140 and Chiron Corporation a corporation having a principle place of business located.....

THE PARTIES HAVE AGREED AND DO AGREE AS FOLLOWS:

1. This AGREEMENT refers to the work of Dr.....  
William S. M. Wold  
relating to adenovirus 11.6K protein that functions in  
promoting cell death.
2. .... agrees to maintain the confidentiality of Proprietary Information discussed during their meetings. Proprietary Information shall not either be used or disclosed to any other person or entity without the written consent of the St. Louis University Medical Center except as may be required under a court order.
3. The confidentiality obligation shall not apply to:
  - a: Information which ..... can establish by reasonable proof was in its possession at the time of disclosure.
  - b: Public domain information or information which after disclosure becomes part of the public domain information by publication or otherwise except by breach of this Agreement.

The term and condition herein are acceptable to both parties of this Agreement as evidenced by the signatures of their authorized representatives:

Signature \_\_\_\_\_

Date: \_\_\_\_\_

Name: Aftab Alam

Title: Director, Biotechnology Transfer Research Center

Organization: St. Louis University Medical Center

Signature \_\_\_\_\_ Date \_\_\_\_\_

Name/Print \_\_\_\_\_ Title: \_\_\_\_\_

Organization: .....

## ADENOVIRUS E3-11.6K PROTEIN AS A CELL DEATH-PROMOTING AGENT

### A. BACKGROUND

The human adenovirus type 2 (Ad2) and Ad5 E3-11.6K protein (MW of 11,600) (Fig. 1) was first identified by our laboratory, using an anti-peptide antiserum to immunoprecipitate the protein from adenovirus-infected cells (Wold et al., 1984). Subsequently, we have prepared three additional anti-peptide antisera, we have isolated mutants that completely delete the gene, and we have constructed a series of about 20 mutants with truncations, small in-frame deletions, and missense mutations in the gene (Fig. 2; Tollefson et al., 1992; unpublished results). Using these reagents, we have shown that 11.6K is synthesized in low amounts during early stages of infection (prior to viral DNA replication), but in large amounts at late stages of infection (Tollefson et al., 1992). We have also shown that 11.6K is an N-linked O-linked integral membrane protein that initially localizes to the endoplasmic reticulum (ER) and Golgi apparatus, but ultimately localizes to the nuclear membrane (Scaria et al., 1992).

We have recently discovered that the 11.6K protein functions to promote cell death (A.E. Tollefson, A. Scaria, and W.S.M. Wold, manuscript in preparation). A representative experiment is shown in Fig. 3. As measured by release of lactate dehydrogenase (LDH), cells infected with wild-type adenovirus (*rec700*) begin to die at 2-3 days postinfection (p.i.), and are mostly dead by 5-7 days p.i. In contrast, cells infected with a mutant (*dl712*) that deletes only the 11.6K gene stay completely alive until 5 days postinfection, and they do not begin to die until 6 days p.i. Similar results have been obtained using, as indicators of cell death, the release of adenovirus from infected cells, trypan blue exclusion, the MTT assay (which measures mitochondrial activity), DNA degradation (agarose gels, DAPI staining, Apo-Tag), and light and electron microscopy. We believe that the function of 11.6K in adenovirus biology is to lyse

cells, thereby allowing adenovirus to be released from the infected cell.

Using our collection of virus mutants in the 11.6K gene, we are currently performing a structure-function analysis of 11.6K. Some of the mutants are shown in Fig. 2. The phenotypes of the mutants are summarized at the right side of Fig. 2. SDS-PAGE analysis indicated that all the mutant proteins are stable with the possible exception of *pm734.1* which only synthesizes residues 49-101 of the 11.6K protein (data not shown). These mutants have allowed us to map the protein domains required to target 11.6K to the nuclear membrane, and for 11.6K to promote cell death. Briefly, residues 41-59, the only hydrophobic domain in the protein, serves as the signal to insert 11.6K into membranes and to anchor 11.6K within membranes, i.e., this domain is a signal-anchor sequence. Residues 63-78, which includes the basic-proline domain (residues 63-74), a domain rich in basic amino acids and proline, is required to target 11.6K *specifically* to the nuclear membrane; mutant proteins that lack these sequences localize to *all membranes*, not specifically to the nuclear membrane. Regarding cell death, residues 1-40 and 71-101 can be deleted without abrogating the ability of 11.6K to promote cell death. Thus, the "death domain" appears to consist of residues ca. 46-60, with help from residues 61-74. However, we emphasize that although we have shown that these sequences are necessary to promote cell death, we have not shown that they are sufficient.

We do not understand the mechanism of action of 11.6K in targeting to the nuclear membrane and in promoting cell death. We believe that the nuclear membrane is the site of action of 11.6K because certain mutant proteins that do not exit from the ER or Golgi are defective in promoting cell death. Future studies in the laboratory will focus on the mechanism of action of 11.6K. This mechanism is not only of fundamental interest, but it may also

elucidate the cellular mechanisms that control cell death; this latter information may allow for novel gene therapy approaches for killing or protecting cells.

## B. PROPOSAL

Since the 11.6K protein can promote the death of adenovirus-infected cells, it has the potential use as a therapeutic agent to kill cells, e.g. malignant cells, in humans. This proposed research addresses two issues that must be resolved in order for 11.6K to be used as a therapeutic agent.

First, although we know that 11.6K promotes the death of *adenovirus-infected cells*, we do not know whether 11.6K can *function autonomously* to kill cells.

Second, since 11.6K functions inside the cell, apparently at the nuclear membrane, a means must be developed to deliver the protein to the cells of interest.

## C. EXPERIMENTAL PROCEDURES

### C.1. Can the 11.6K protein function autonomously to kill cells?

In order to answer this question, we have attempted to isolate human A549 cells stably transfected with the 11.6K gene. Several neo<sup>R</sup>-resistant cell lines have been obtained. These cells express only low levels of 11.6K, and the 11.6K protein is localized in the Golgi but not the nuclear membrane. Since we were not able to obtain cells that express high levels of 11.6K, or cells wherein 11.6K is localized to the nuclear membrane, these experiments suggest, but do not prove, that 11.6K can function autonomously to kill cells. We propose two approaches to address this question in more detail.

#### C.1.a. Tet-inducible vector.

We have obtained the *tet*-inducible vector system from Hermann Bujard

(Gossen and Bujard, 1992). In the *tet* system, the tetracycline repressor (tetR) was fused to the transactivation domain of VP16. In the absence of tetracycline, the fusion protein will bind to *tet* operators and will transactivate a promoter consisting of *tet* operators plus a TATA box. In the presence of tetracycline, the tetR-VP16 fusion protein does not bind to *tet* operators, and the promoter is silent. The major advantages of this system are that, upon removal of tetracycline, the gene is induced by up to five orders of magnitude. Also, induction is rapid, being >20% in 4 h and 100% in 12 h. Finally, the low amount (0.1  $\mu$ g/ml) of tetracycline required to keep the gene silent is unlikely to affect other properties of the cell. We will clone 11.6K into the *tet* vector, develop cell lines, and examine whether 11.6K is induced by removal of tetracycline, and whether 11.6K promotes cell death following induction. Cell death will be measured by release of LDH, trypan blue exclusion, the MTT assay, and microscopy.

If 11.6K alone does promote cell death, then we will proceed with the experiments described in Section D below. If 11.6K alone does not promote cell death, then we will proceed with the experiments in Section C.3.

#### **C.1.b. Adenovirus vector.**

There are two general classes of adenovirus vectors, nondefective and defective for replication in cultured human cells. The nondefective vectors generally have the E3 transcription unit (Fig. 4) deleted and replaced with the transgene. The E3 genes are not required for virus replication in cultured cells or in the lungs of hamsters or cotton rats, so these vectors are able to replicate. However, the E3 genes function to block the immune and inflammatory response to virus infection, so the E3-deleted vectors are more pathogenic than wild-type adenovirus.

Defective vectors generally have the E1A and E1B regions deleted and replaced with the transgene, and they have the E3 region deleted in order to increase the amount of foreign DNA that can be inserted into the viral genome (only 105% of the genome can be packaged). The E1A and E1B genes (E1B-55K) are essential for virus replication. E1A proteins are required to efficiently induce transcription of the other adenovirus transcription units. The E1B-55K protein is required for efficient transport of viral "late" mRNAs from the nucleus to the cytoplasm. Since the defective vectors lack E1A and E1B, they cannot replicate in ordinary cell lines or in animal models. The vectors can, however, replicate well in 293 cells, a human cell line that provides the E1A- and E1B-encoded proteins *in trans*.

Although E1A is required for *efficient* induction of transcription of the other adenovirus transcription units, transcription of these genes, including late genes, can occur at low levels in the absence of E1A. Indeed, recent animal model studies and Phase I human trials have indicated that defective adenovirus vectors do elicit an inflammatory response, presumably due to low level expression of adenovirus proteins.

Since we eventually hope to design an adenovirus vector to promote cell death, it will be important to limit the infection *in vivo* to the target tissue, and to minimize infection of healthy tissue. Therefore, the vector should probably be defective. The expression of the 11.6K protein could be limited to the tumor by direct injection into the tumor, or by use of a tumor-specific promoter to drive expression of the 11.6K gene. It is unclear whether the E3 genes should be included to minimize the normal inflammatory response (probably not), or excluded in order to maximize the response; this presumably would depend on the amount of infection of healthy tissue.

The design of an optimal vector is a topic of future experiments. At the present, we will use a simple "first generation" vector to address whether 11.6K expressed essentially alone can kill cells. This experiment differs from that described in Section C.1.a. because the vector will express other adenovirus proteins at low levels.

Our vector will have an Ad5 backbone, and it will be deleted in the E1A, E1B, and E3 regions. The 11.6K gene will be inserted into an expression cassette wherein transcription will be driven by the cytomegalovirus immediate early promoter, and the pre-mRNA will be processed using SV40 polyadenylation and splicing signals. The expression cassette will be inserted into the E1A/E1B region (Fig. 4), and plaques will be picked on 293 cells. Plaques of vector expressing 11.6K should be larger (more cell lysis and virus spread) than plaques from vector lacking 11.6K. Plaques will be expanded into virus stocks, and high-level expression of the 11.6K protein will be confirmed. Cultured human and mouse cells will be infected with the vector, and cell death will be monitored as described in Section C.1.a.

If 11.6K expressed from the adenovirus vector kills cells, then we will proceed with the experiments in Section D. If cells are not killed, then we will perform the experiments in Section C.3.

## **C.2. Mapping the minimal domain of 11.6K that is sufficient to promote cell death.**

Our studies with 11.6K mutants have indicated that a protein consisting only of residues 41-101 (i.e. lacking residues 1-40) localizes quite efficiently to the nuclear membrane and is about 50% as effective as the wild-type 11.6K protein in promoting cell death. A protein lacking residues 79 to 101 is completely wild-type for localization to the nuclear membrane and promotion of cell death. These results suggest that a version of the 11.6K protein consisting



only of residues 41-78 might be sufficient to promote cell death. It is important to determine whether this is so, because such a peptide, synthesized *in vitro*, might by itself be sufficient to promote cell death (e.g. by injecting the peptide into tumors). Also, such a peptide might be less immunogenic than the entire 11.6K protein. We propose to construct an adenovirus mutant that expresses residues 41-78 and determine whether this protein can promote cell death. This mutated gene will be inserted into the *rec700* background, as we have done for all our other 11.6K mutants, and it will be expressed from the *tet*-inducible vector.

### **C.3. What is required in adenovirus-infected cells in order for 11.6K to promote cell death?**

If the experiments described in Section C.1. indicate that 11.6K alone cannot promote cell death, then some other adenovirus gene product or some unknown aspect of the adenovirus productive infection must be required in order for 11.6K to function. The following experiments will be performed to address these issues.

First, we will determine whether 11.6K overexpressed during early stages of infection can promote cell death. The 11.6K gene will be built into our mutant, *d17001*, which lacks the entire E3 region but expresses all other adenovirus genes. We are using this mutant as a vector to express the E3 proteins individually (Ranheim et al., 1993). Cells will be infected with the *d17001*-11.6K vector, maintained in cytosine arabinoside in order to inhibit viral DNA synthesis and keep the cells in the early stage of infection, and cell death will be monitored.

Second, we will attempt to map the adenovirus early protein(s) that must collaborate with 11.6K in order for 11.6K to promote cell death. We will infect cells with mutants that lack the E4 region, the E1B region, and the E1A region. We already know that

this putative "collaborating" protein is not encoded by the E3 region. These experiments are similar to those we performed to map genes that function to sensitize cells to tumor necrosis factor (Duerksen-Hughes et al., 1989) and to down-regulate the epidermal growth factor receptor (Carlin et al., 1989). If we determine that an adenovirus late gene encodes the "collaborating" protein then we will attempt to map the gene using available temperature-sensitive mutants.

#### **D. POTENTIAL FUTURE EXPERIMENTS**

The following is a brief description of possible future experiments. Which experiments will be attempted will depend on the results of the experiments in Section C. Most or all of these experiments will probably require additional funding.

##### **D.1. Design of an optimal vector to deliver the 11.6K protein to cells.**

We will attempt to optimize expression of 11.6K in our adenovirus vector, and we will construct vectors with tissue-specific promoters. We will explore whether the E3 region should be included in the vector, and whether a nondefective vector might be useful.

We will also attempt to express 11.6K from a retrovirus vector. Since retrovirus vectors only infect dividing cells, such a vector that expresses 11.6K might be particularly useful to treat brain tumors.

##### **D.2. Delivery of the 11.6K protein by direct injection.**

Since 11.6K has an internal signal-anchor sequence and it has only a short sequence that is C-terminal to the signal-anchor domain, 11.6K may be able to insert into membranes in a posttranslational manner. If so, then liposomes could be an ideal mechanism to deliver the protein to the interior of cells. The 11.6K protein might integrate into the

membrane of the liposomes, become fused to membrane vesicles within cells, and then be targeted to the nuclear membrane via its specific nuclear membrane localization signal. We will attempt this experiment using 11.6K purified from adenovirus-infected cells, where 11.6K is made in large amounts. Alternatively, 11.6K could be obtained from bacterial or baculovirus vectors.

If the experiment described in Section C.2. results in the identification of a minimal domain for 11.6K to function, then the minimal peptide will be synthesized *in vitro*. The peptide will either be incorporated into liposomes or added directly to cells, and cell death will be monitored.

#### **D.3. Coupling of the 11.6K protein to a ligand.**

In one of our adenovirus mutants (*dl718*), the stop codon for 11.6K is deleted; this results in the synthesis of a fusion protein consisting of residues 1-87 in the 11.6K protein fused to residues 14-91 of the E3-10.4K protein. This fusion protein functions nearly as well as wild-type 11.6K in promoting cell death. This suggests that 11.6K could be fused at its C-terminus to some ligand, e.g. EGF, and still remain functional. If so, then the ligand could be used to target 11.6K to specific cell types that express the receptor for the ligand. Thus, the experiment would be to express the 11.6K-ligand fusion protein in bacteria or insect cells, purify the protein, add the protein to the cells, and determine whether the protein is efficiently internalized into cells that express the ligand receptor, and whether the protein promotes cell death.

#### **D.4. Animal models.**

We have found that 11.6K does not localize to the nuclear membrane in

adenovirus-infected mouse or rat cells. This may imply that 11.6K must interact with a human protein in order to promote cell death. Lack of 11.6K function in mouse or rat cells is in one respect unfortunate because an animal model will be necessary to determine whether 11.6K can, for example, cause regression of tumors *in vivo*. We will infect cells of different species, e.g. cotton rat, hamster, and monkey, and determine whether 11.6K localizes to the nuclear membrane and promotes cell death. It may also be possible to examine human tumors growing in immunoincompetent mice. When we find a species where 11.6K is able to promote cell death, then experiments will be done in this animal model.

## REFERENCES

Gossen, M., and Bujard, H. 1992. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc. Natl. Acad. Sci. USA* 89, 5547-5551.

Ranheim, T.S., Shisler, J., Horton, T.M., Wold, L.J., Gooding, L.R., and Wold, W.S.M. 1993. Characterization of mutants within the gene for the adenovirus E3 14.7-kilodalton protein which prevents cytolysis by tumor necrosis factor. *J. Virol.* 67, 2159-2167.

Scaria, A., Tollefson, A.E., Saha, S.K., and Wold, W.S.M. 1992. The E3-11.6K protein of adenovirus is an Asn-glycosylated integral membrane protein that localizes to the nuclear membrane. *Virology* 191, 743-753.

Tollefson, A.E., Scaria, A., Saha, S.K., and Wold, W.S.M. 1992. The 11,600-M<sub>w</sub> protein encoded by region E3 of adenovirus is expressed early but is greatly amplified at late stages of infection. *J. Virol.* 66, 3633-3642.

Wold, W.S.M., Cladaras, C., Magie, S.C., and Yacoub, N. 1984. Mapping a new gene

that encodes an 11,600-molecular-weight protein in the E3 transcription unit of adenovirus 2.

*J. Virol.* 52, 307-313.

## FIGURE LEGENDS

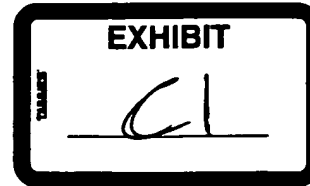
Fig. 1. Sequence of the Ad2 E3-11.6K protein. The protein becomes integrated into membranes via its internal signal-anchor domain at residues ca. 41-59. The protein is oriented in the membrane with the NH<sub>2</sub>-terminus in the lumen and its COOH-terminus in the cytoplasm or nucleoplasm (the protein ultimately localizes to the nuclear membrane, but we do not know whether it is the inner or outer nuclear membrane). The site of N-glycosylation is indicated (NTT), as are the major sites of O-glycosylation. A domain rich in basic amino acids and prolines is indicated.

Fig. 2. Schematic representation of the Ad2 11.6K protein, as well as a subset of the mutants constructed in the 11.6K protein. For the wild-type protein (top figure), the major and minor O-glycosylation sites are shown, the N-glycosylation site is shown, the methionines at positions 1, 41, 49, and 56 are shown, the signal-anchor domain is shown, and the basic-proline domain is shown. Also shown is the orientation of the protein in the membrane, with the NH<sub>2</sub>-terminal region in the lumen of membranes, and the COOH-terminal region extending into the cytoplasm and/or the nucleoplasm. The deletions shown for the various mutants are indicated by the schematic. The two right columns in the figure indicate the approximate phenotype of the mutants with respect to their ability to promote cell death, as a percentage of the wild-type protein, and their ability to localize to membranes. Membranes shown in italics are the major sites of localization. NM refers to nuclear membrane, G refers to Golgi. The protein depicted at the bottom of the figure corresponds to the putative proteolytic processing product that arises following initial synthesis of the 11.6K protein.

Fig. 3. A cell viability assay for cells infected with *rec700* (wild-type) or two mutants,

*pm712* and *pm734.1*, which lack a functional 11.6K gene. A549 cells were infected with the viruses, then at different days postinfection cell lysis was measured based upon the release of lactate dehydrogenase (LDH) into the culture medium. The experiment shows that cells infected with *rec700* die (i.e. release LDH) much more rapidly than do cells infected with the two 11.6K mutants.

Fig. 4. Schematic of the Ad2 genome. The genome is a linear duplex DNA of 36,000 base pairs. *r* and *l* refer to rightward and leftward transcription, respectively. The split arrows indicate the spliced structures of the mRNAs (exons are shown). 289R, 19K, IIIa, etc. refer to proteins. E1A, E1B, E2 (A and B), E3, E4, and L1 (early) are "early" transcription units that are expressed prior to viral DNA replication. L1-L5 are families of "late" mRNAs expressed in the major late transcription unit. The 11.6K protein is translated from one of the E3 mRNAs at early stages of infection. At late stages of infection, 11.6K is translated from an mRNA that contains the major late tripartite leader (Tollefson et al., 1992).



Kostya, January 30

Project: Develop replicative vectors for cancer gene therapy.

### Progress achived:

1) 3 rounds of transfection were performed with p626 (pBHG11 with E4 promoter substituted for for CMV promoter). No plaques were obtained with this construct or pBHG11 as a control. I have prepared new stock of pAV56 (left-arm plasmid) and repeated the experiment 3 times more. Now waiting for results.

2) The plasmid containing E4 promoter- SPB promoter substitution was constructed (p82). It contains DNA of dl309 from BamHI to NdeI site (E3 region from dl309) and other part to the end of genome from pBHG11 with substitution of E4 promoter. 1 cotransfection experiment was done recently with 1101/1107 cut with EcoRI and pCMV/TTF. *Get some TTF at present*

3) Cotransfection of 1101/1107 cut with EcoRI and p54. (Lynda's plasmid pdILKH Bam-end with insertion of Ad5 ADP gene) was done. Up to now (26 days) there are no plaques with either 1101/1107/ EcoRI or with 1101/1107/EcoRI+ Lynda's plasmid (parental plasmid without insert of ADP). *With p54 there are 7 plaques* The plaques have been picked up and initial stocks of viruses prepared. Now the work is in progress to infect monolayers and characterize the genomes of this viruses. *appear in 2 weeks.*

4) I failed 2 times to construct the large plasmid with mutated gp19K on dl309 background. Now *2asm* repeating the cloning. *BDP inserted in XbaI site. Not start in 70.*

### Proposed work:

1) To join Carol's and Mohan's work on 1101/1107 and pm975. I presume it would be interesting to do the same work on primary lung epithelial cells (GTI). SAEC - normal human lung small airway epithelial cells. *GTI get cells from GTI.*

2) Start transfection experiments on Mohan's cell line (293 transformed with pCMV/TTF) to obtain the virus with E4- SPB promoter substitution. Characterize Mohan's 293-TTF cells on their ability to transactivate SP-B promoter (transfection and IP with anti-E4 ORF6 antibodies).

3) Start the work to obtain the plasmid with E1 on 1101/1107 background? Make the point mutation as in pm975? Try to substitute E1 promoter for SPB? Enlarge the CR2 deletion?

*GTI - infect cell line.*



Jan 31, 97

2

## Outline of Mohan's project:

### 1. C. F. project:

a) plasmid p $\Delta$ E1splA/3.7SPC Rep78 has ~2900 bp deletion. (Gautam's plasmid).

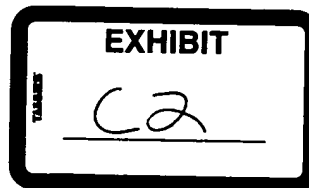
b) Construction of p $\Delta$ E1splA/3.7SPC Rep78 is in progress: Transferring the 3.7 SPC/Rep78 fragment to p $\Delta$ E1splA plasmid. Next week SPO/Rep78.

c) Preparation of <sup>psal assay</sup> high titer BHL E3TR LacZ virus.  
 $1.4 \times 10^9$  pfu/ml. Done with 40 dishes.

Looks like virus does not grow well.  
 Try again in KB cell/E1 growing in spinners.

KB/E1 - looks like may grow in spinner.

### 2. Cancer project:



#### a) Experiment 1:

a-1): Growing HEL 299 cells infected with d1101/110:  
 Showed CPE ~ 12 days.  
 d1309 showed CPE on 3<sup>rd</sup> day.

a-2): Growth arrested HEL 299 cells infected with d1101/1107 did not show CPE, till 17<sup>th</sup> day. d1309 showed CPE on 8<sup>th</sup> day.

a-3): IF showed strong presence of fiber and DBP in ~ 50% of the growing cells (infected with dl1101/1107) on 14<sup>th</sup> and 17<sup>th</sup> day.

b) Experiment 2:

b-1): pm 975 infected growing HEL 299 cells showed CPE on 3<sup>rd</sup> day; dl 309 also showed CPE on 3<sup>rd</sup> day.

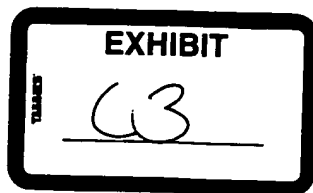
: pm 975 and dl 309 killed all the cells ~ 6<sup>th</sup> day.

, dl 1101/1107 showed CPE beginning ~ 12-14 days.

b-2): Till 10<sup>th</sup> day <sup>→ 17 days - still still no CPE</sup> no visible CPE was seen. <sup>Cells frozen. Next week plaque assay.</sup>  
in pm 975 and dl 1101/1107 infected growth arrested cells.

dl 309 showed CPE on 8<sup>th</sup> day.

b-3): Growth curve = Growing and growth arrested viral infected cells are being frozen for plaque assay.



c) Experiment 3:

c-1) HEL 299 growing cells are infected with PM975; dl 1101/1107: dl 309 and mock, at MOI of 100 pfu and 500 pfu/cell. 3<sup>rd</sup> of infection, at 100 pfu PM975 showed moderate CPE and ~90% CPE in 500 pfu infection. No CPE is seen in dl 1101/1107 infected cells at 100; and 500 pfu/cell. (7<sup>th</sup> day) dl 309 showed CPE on 3<sup>rd</sup> day (100 pfu) and 90% CPE in 500 pfu infection.

Growth arrested cells will be infected today.

Plaque assay not week.

EXHIBIT

C4

Gene Therapy - Group Meeting 9/10/01

Took 1 hr 20 min

Karoly 2/17/87

Did not  
make happen  
ID, 14.7 in  
EIA

E3 viruses

- I have made GGL stock of pGlu (D-) 14.7 and NPGK pp18k ap
- titring ID under process (NPGK pp18k ap  $\approx 10^9$ )
- going to make Ad lac Pol RID and Ad lac Pol 14.7  
RID in E3 region. No band in Cell.

Bai - CMV-14.7 CMV-RID. Have done. Make virus.

FasL project

FasL  
20-transfected 293  
10.4/10.5 plasmid  
pCI (Bai made)  
cells died.

- I have cloned mouse FasL in pCI, then the CMV-mFL-polyA cassette in a left end plasmid containing p-Gal and VARKA I-II
- Transfected this construct in 293  $\rightarrow$  the cells died
- I could not show mFL in a transient transfection assay (293 cells, Western blot with anti human FasL) but the protein could be detected in an in vitro transcription-translation reaction using the same plasmid and antibody
- Transiently transfected MCF-7 and MCF7 Fas cells die at the same rate. No cyclo treatment is needed
- I am going to try MCF-7 ConA first in transient transfections, then make a cell line. This cell line can be used as an "activated CTL" in Fas experiments
- I am making a ConA-293 line

EXHIBIT

C5

Cancer Therapy project

- It seems that the V138 I use is too high passage. Only a proportion of the cells divide, the majority is senescent. I have ordered a new batch from ATCC.

February 14, 1997 Kostya

- 1) Up to now I have no plaques after transfections with p626 (E4  $\rightarrow$  CMV promoter substitution). And I have no plaques with p84G11 as a control.
- 2) I am preparing now DNA from cells infected with 10 plaques resulting from cotransfection of 1101/1107 DNA cut  $EcoRI$  with p54 (Lynda's deletion of E3, insert of ADP gene).
- 3) I have plaques after cotransfection of 1101/1107 DNA cut  $EcoRI$  with p82 (E4  $\rightarrow$  SP-B) promoter substitution. Control (1101/1107 cut with  $EcoRI$ ) to experiment ratio is 3:10 now but in dishes with p82 plaques are still appearing (3 weeks).
- 4) I am working on Moken's cells (293 CMV/TTF) have done one cotransfection of 1101/1107 /  $EcoRI$  p82 on these cells.
- 5) Cloning experiments to obtain large plasmid suitable for cotransfection with gp191C mutated are in progress.

EXHIBIT

C6

Feb 14, 97

# Outline of Mohan's Project:-

1. C.F. project:
- RNA analysis of Rep78 antisense
  - Hist - photograph of passage (1st x 10<sup>9</sup>) fixed on 293 cells. 80 dishes
  - a) Screening colonies for 3.7SP4/Rep78 insert in pXELSP1A.  
36 colonies are under screening; some colonies have the insert; screening more colonies & characterization
  - b) SPB promoter = PCR; strategy to determine point of Rep78.

Mohan - KB/E1A plasmid - doing ok. Still in tube (60 ml). Not in spinner yet.  
293 suspension culture. Grow slow. Form clumps at  $5 \times 10^5$  cells/ml.

## 2. Cauler project:

### Experiment #3:

Growing HEL 299 cells: 14<sup>th</sup> day (2/14/97)

- a) pm 975 (100 + 500 pfu) killed all the cells.
- b) dl 1101/1107 (100 pfu) + CPE starts in 500 pfu - shows CPE. (better than 100 pfu)
- c) dl 309 (100 + 500 pfu) killed all the cells.

Growth arrested HEL 299 cells: 7<sup>th</sup> day

- a) No CPE in dl 1101/1107 (100 + 500 pfu);
- b) No CPE in pm 975 (100 pfu);  
CPE shows in 500 pfu. Same as 309 at 500 PFU.
- c) No CPE in dl 309 (100 pfu)  
CPE shows in 500 pfu.

### Growth Curve:-

Dishes are processed; going to do plaque assay

EXHIBIT

C7

Kordy 2/14/97

E3 viruses

- Titer of N PGK pp19kex is  $1.37 \times 10^9$
- $\beta$  Gluc(D-) 12.7 is less than  $10^7$  (?) (will repeat titer exp.)
- We are trying to make virus with the plasmids made by Bai
- We are assaying for expression of 12.7 and R10 in a Western

FosL project

- I tried the infection-transfection experiment twice. I failed both attempts because the virus killed the cells before I could transfect. I used 50 pfu/cell (233), 20  $\mu$ g/ml AraC. The cells were rounded up after 18 h (1st exp.) and 7 h (2nd exp.) 5 pfu. MCF7 cells. FosL supernatant. 293/CmV cell line.

Cancer Therapy project

I have got WI38 from ATCC. I am growing them up. Got cell proliferation bet-stains for BuddR.

EXHIBIT

C8

Kostya

1) I have made and analyzed by restriction and  
PCR ~~test to~~ Hint DNA preps from S' of 10 plaques  
that I had after p54 <sup>(Lynnda's plasmid + ADP)</sup> cotransfection with 101/109/R1.  
2 plaques were wt with addition of something  
that I suggest is defective version of recombinant  
virus. 3 other plaques contain E3 from p54.

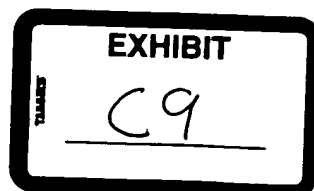
2) I'm <sup>Have 10 plaques - half small, half very small. One month. The transfection was complicated.</sup> picking up plaques resulting from transfection  
of p82 (E1  $\rightarrow$  SP-B promoter substitution). <sup>co-transfected Cx41-  
cut 8/10/11/07  
also transfected TFF1.</sup>  
Expt is being repeated - expt is 3 weeks old; Had same plaque.

3) Failed to construct large form of gp191c \*  
plasmid due to contamination of competent cells  
with something AmpR. Now prepared new batch  
of competent cells, about to repeat cloning.

Ad/ADP recombinant in "Lynnda's plasmid, a E3, + C1. Delitem extending like p3H411.  
Has 4 plaques, 2 are recombinant (Hint, PCR), 2 seem to be mix of wt and recombinant  
with a deletion. These plaques give strange appearance, as if they are secreting  
a factor that kills surrounding cells - ~~trans~~  
Lynnda's plasmid - not even sure that E4 is present.

Kostya - well plaque - plenty the "mutant" and "mixed" plaques.

The two mutant plaques are very "slow" - may not be good for further virus.





New Energy Group Meeting 972221

(PCI) CMV-internal gene - SV40 poly A  
Carolyn 2/21/98

### ES viruses

- Western with the pCI RID and CMV-14.7 plasmids was negative - I am doing an IP in pA1501A
- These are the constructs that Dai made.

### FasL project

- The shudded mFasL is not toxic
- I am trying to make virus with mFasL in left-end plasmid and d17001

It is known that the human FasL is <sup>shudded</sup> toxic, the mouse FasL is <sup>very fast</sup> not.

All MCF-7 cell lines die when transfected with vector/FasL except the one in Cunt.

They die in one day.

25-50% of the cells die.

Will do exp in MCF-7/147K cell line.

Try any MCF-7 cell line expressing RID or RIDP?

Could try to repeat this exp in A549, even if transient transfection is poor.

Karl-making 793 cells expressing Cunt.

MCF-7/CuntA/FasL - have colonies.

- transfected with RID + LDNGFR, purify, then transfected with FasL.

EXHIBIT

C10

## Mohan's project.

Feb 21, 97

## 1. CF. Project:

a) Final plasmid p $\Delta$ ELSP1A/3.7SPC Rep78 has been constructed; plasmid preparation is under progress.

## 2. Cancer project:

## Experiment #3:

Growing HEL 299 cells 21<sup>st</sup> day

a) d1101/1107 shows better CPE in 500 pfu than 100 pfu.

b) pm 975 & d1309 killed the cells.

Growth arrested : 14<sup>th</sup> day

a) NO CPE in pm975 (100 pfu).

At 500 pfu at 14<sup>th</sup> day cells are killed.  
BPE starts at 3rd day in confluent area. Cells died at 12 days.

b) NO CPE in d1101/1107 (100 & 500 pfu)

Growth Curve:-

pm 975 - infected G & GA. HEL 299 cells:

Growth Curve is done; waiting for plaques.

Will need to do growth curve for d1101 - will do next week.

Northern blot will be done for Rep78 antisense cell line  
Trying to clone SPB/Rep78 into p.

EXHIBIT

C11

12-28-91 Kostya

Need to do Hirt on some of these plagues.

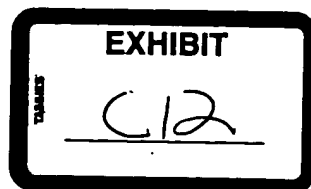
1) I have analyzed by PCR 8 of 10 plagues which I had after 1101/1107 cut with EcoRI + p82 (E4 → SP-B promoter substitution, E3 from dl 309). They all contain wt E4 prom. And I have no up to now plagues with p626 (E4 → CMV promoter substitution, E3 from pBK611). *Determine RHE sequence of Kruppa's plasmid.*

1) I am going to put different variants of E3 that I have in ~~at~~ <sup>small</sup> plasmids (dlE3 X60 + ADP, dl 309 with gp19K unmutated, wt?) into Linda's plasmid with which I have obtained a recombinant virus.

1) I would ~~propose~~ to start the work toward E1 promoter deletion and substitution with SP-B or any other tissue-specific or tightly regulated promoters. As alternative we can try to substitute E2A promoter.

1) Growing up ADP mutants vectors.

GLAST lipoxigenase - 3 diff species.



Mohan,

Feb 28, 97,

## 1 C.A. Project:

- a) Preparation of pSEISPIA/SPC Rep7K is progressing. Some problem is CsCl banding. Preparing another prep. Saw 4 bands - good band.
- b) Construction of SPB- Rep7K plasmid.  
: partial digestion of p3.7SPC/3540 with HindIII -

EXHIBIT

C13

## 2. Cancer Project:

## Experiment # 3:

Growing HEL 299 cells 28<sup>th</sup> day1101/1107  
good CPE  
at 500 pfu

- a) DI 1101/1107 shows CPE in 500 pfu and 150 pfu

Growth arrested cells:- 21<sup>st</sup> day.

- a) pm 975 at 150 pfu shows ~ CPE?
- b) NO CPE in DI 1101/1107 (150 & 500 pfu)

Growth Curve:- pm 975 150 pfu. On 293 cells.

Growing:

3 <sup>rd</sup> day	= $7.1 \times 10^4$ pfu/ml
5 <sup>th</sup> day	= $1.3 \times 10^5$
6 <sup>th</sup> day	= $1.1 \times 10^5$
7 <sup>th</sup> day	= $7 \times 10^6$

Growth arrested:

1101/1107

assay has been

started

Need to strain next week.

Need to check 1st & 3rd day.

10 <sup>th</sup> day	= $1.2 \times 10^5$ pfu/ml
14 <sup>th</sup> day	= $3.2 \times 10^5$
19 <sup>th</sup> day	= $1.8 \times 10^5$

K13/E1A are growing. G419 resistant. Not proved that 293 cells freeze from microtub.

Keroly 2/28/9

E3 viruses

3. transfection - IP with Bai's constructs: I could IP 14.5 (and going. No plasmid yet) Co-IP 10.4(?) from an in vitro translated sample. After one day exposure the transfected samples did not show up. The 14.7 plasmids were negative even in the in vitro translated samples. Sequencing is under process. No 14.7<sup>th</sup> made CCL stocks of Krigen's two plasmid p-Gal-PGK14.7ap and p-Gal-R10ap. I am going to try to make virus with them.
- 1101, 118 day pi  
B4H8-11  
B4H8-10  
TAY  
H610-plasmid on the 14.7 day

Fest project

- Have McF-7-Fest - I have transfected R10 and mFest (both in pCI vector) in 293 cells. Result is due this evening tomorrow morning.
- I have infected 293 cells with the CPE stock from the d17001 infection - LE mFL transfection experiment.

Cancer Therapy project

- I use the new K138 cells for a new round of infection with 1101/1107

I need to call Bruce Trapnell. What is real status of money.

EXHIBIT

C14

3/7/97 Kostya

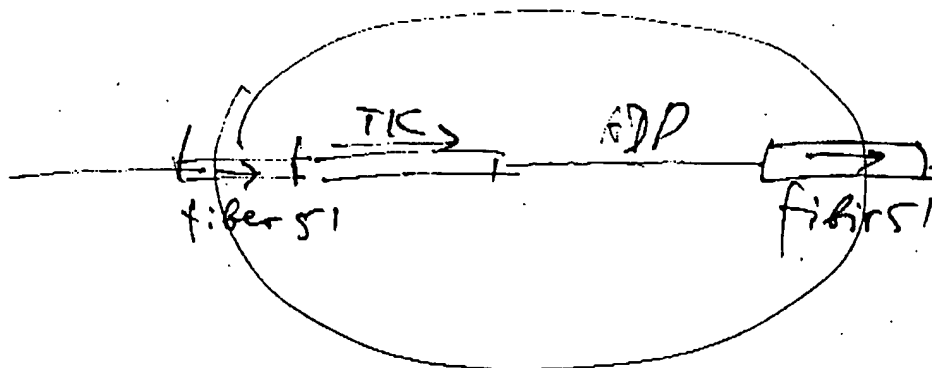
- 1) Cloning of ITR PCR product to p82 (EU-SPB promoter plasmid) is in progress.
- 2) I have a problem with TK, cannot PCR it from Linda Morrissey's plasmid. If it is possible, I would like to have a plasmid with HSV-1 TK gene from GT1.

- 3) Plaque assay of S44 (vims with Lynda's E3 deletion + ADP insert) on 293 cells.

(309, 1101/1102) (wt control) <sup>Lynda's deletion</sup>  
 12.5K nt6 L4 polyA  
 mutate retains

(S44) develops plaques a bit slower than wt, plaques appeared 1-2 days later than in wt, day 16 - plaques are ~ 8-10 mm in diameter.

(7001) - plaques are tiny (1-2 mm) at day 16.



EXHIBIT

C15

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



03-07-97

Kostya

1) i) Results of cotransfection 1101/1107 /EcoRI + p54 (dlE3 + ADP) → 4 more plaques were analyzed. 3 of them contain recombinant genomes.

ii) Subcloning of dl 309 E3 with gp19K mutated and dlE3 Xba + ADP into Linde's plasmid is in progress. When the plasmid with 19K\* is made I'm going to insert HSV-1 TK into PacI site created in gp19K gene.

2) i) No plaques with p626 (pB611 E4 promoter → CMV promoter). No plaques with pB611 as a control.

ii) 4 more plaques resulting 1101/1107 /EcoRI + p82 (E4 → SP-B promoter) were analyzed. All contain wt genomes.

iii) In 2 repetitions of cotransfection 1101/1107 with p82 (E4 → SP-B promoter) no plaques neither in control nor in experiment. 2nd experiment was done on Moken's 293/CMV-TTF cells.

Now repeating the cotransfection.

iv) Sequencing of Nippen's plasmid p21 (E4 promoter dl) is in progress to prove that right Ad ITR is intact in the plasmid.

3) Growing initial stocks of the recombinant virus with dlE3 + ADP.



Kathy 3/7/97

E3 viruses

- I have transfected p-Gal PGK 147 ep and p-Gal PGK R1D<sub>9</sub> (with B4611) I am waiting for plaques.
- Still no plaques in Bai's constructs. Now 3 weeks old.

FasL project

- The pC1R1D construct did not protect against killing induced by pC1mFasL transfection (293 cells)

Exp 1 - Transfect with 5  $\mu$ g R1D

- wait 24 h

- Transfect with 5  $\mu$ g mFasLExp 2 - Transfect with 5  $\mu$ g R1D

- wait 24 h

- &gt; plot the cells

- wait 24 h

- transfect with 0.5, 1, 2, 5  $\mu$ g mFasL

- The MCF7 14.7 cell line (Terry) is not protected from killing with mFasL.

- I am trying to show functional R1D in transfected cells (IP, IF)

- I am ~~also~~ trying to show 14.7 in Terry's line (IF).

- I am expanding colonies isolated from 293 cells and MCF7 control mFasL transfections

- I am growing up virus (putative mFasL-p-Gal 7001) isolated from blue plaques.

Cancer therapy project

- 11/01/07 on U138

growing 10th day, arrested 7th day - no difference (no CPE). It seems that there might be a diff.

EXHIBIT

C17



Mohan, March 7, 97

## 1. C.F. Project:

APV-ITR - KB/EI growing  
(PH8 added)

- Preparation of plasmids pDE1sPIA/SPC Rep78 and pB1610: Under dialysis.
- Construction of SPC-Rep78 plasmid - partial digestion  $\rightarrow$  preparative scale.
- RNA blot: For antisense Rep78 RNA in progress.

## 2. Cancer Project:

## Experiment #3:

Growing HEZ 299 cells 35<sup>th</sup> day

- DI 1101/1107 shows CPE in 100 + 500 pfu.

Growth arrested: 28<sup>th</sup> day

- pm 975 at 100 pfu shows CPE.
- No CPE in DI 1101/1107 (100 + 500 pfu)

Growth Curve: DI 1101/1107 (100 pfu) Total cell + supernat

Growing:

5 <sup>th</sup> day	$1.4 \times 10^4$ pfu/ml
15 <sup>th</sup> day	$4 \times 10^4$
24 <sup>th</sup> day	$1.3 \times 10^6$

Growth arrested:

5 <sup>th</sup> day	$1.1 \times 10^3$ pfu/ml
10 <sup>th</sup> day	$1.4 \times 10^3$
19 <sup>th</sup> day	$8.4 \times 10^3$
23 <sup>rd</sup> day	$5.6 \times 10^3$

DO 309  
~~4~~

pm 975 arrested  
growth  
Day 1 + 3  
will be staining  
next week.

Growth Curve: pm 975 growth arrested days (1 + 3) are titrated.

EXHIBIT

C18

TAKES

## I E3 region:

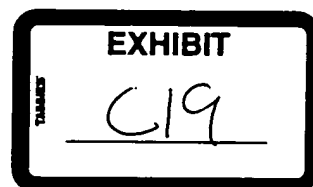
- i) Initial stocks of the viruses with dlE3, ADP insert are prepared. Plaque assay on 293, A549 cells with 1101/1107, dl309, ADP<sup>-</sup> viruses as controls is to be started.
- ii) Two large plasmids suitable for cotransfection with viral DNA based on Lytle's plasmid with E3 a) from dl309 with gpi9K unnoted b) dlE3 Xba + ADP<sup>RO2</sup> are made. CsCl preps of these plasmids are done. Cotransfection of these plasmids with 1101/1107 DNA / EcoRI is in progress. Will do one in TK gene, and PacI site.

## II E4 - SP-B promoter:

i) There were 4 plaques which are developing CPE very slow in plate wells (Incomplete CPE in 3 weeks).

ii) Alternative project: E1 → SP-B (SP-C) promoter substitution. There was an article by David Solnick with E1 → MLP substitution (deletion E1 promoter -45 → +8 relative to E1 cap site).

However, I would propose to try to express E1A under the control of SP-B or SP-C in place of standard E1 deletion. Advantages: a) E1B would be completely deleted in the virus b) It is possible to use 13S cDNA (express only 289 aa E1A)



Fasl project

Kondy 3/14/

- I did an IF with A548 and 293 cells transfected with pCDNA3 RID $\alpha$ , pCDNA3 RID $\beta$  (alone and together) and pCIRID. The results are inconclusive. I could see <sup>strong</sup> RID $\beta$  expression where it was present, but the localization was mostly Golgi, even if RID was supposed to be there. (This was not easy to judge in 293. The IF did not work in A548). The EGF internalization did not work because of the antibody. I am going to repeat this experiment in MCF-7.

- I have labeled IP RID $\beta$ , look for co-IP of RWR.
- I have labeled extract from the same transfection, I have not processed them yet.
- Fasl virus: I am doing a plaque assay with the isolated, blue" viruses.

Startings to test 993/Ornithine. Enough antibody? Transfect: Fasl-seu if possible.

Cancer Therapy project

- I am doing an IF  $\rightarrow$  DAPI, <sup>Blot incorporation</sup> with 1101/07 infected U138
- Growing U138 infected with 500 pfu/cell 1101/07 shows no real cpe on day 15.
- Growing U138 infected with 100 pfu/cell, 175 shows cpe on day 3.

1101/1107 does not grow on primary cells. Perfect.

EXHIBIT

C20

Gene Therapy Group Meeting 970314

Mohan, 3/14/97

1. C.F. Project:

- a) pDEISPIA/3.7 SPC Repts has one additional Koz site;  
Co-Transfected with pBM10 in 293 cells.  
Give to Tolra - Western for E1A and E1B-19K.
- b) KB MMT E1 Spinner cells, frozen same, RTV promoter. Parallel =  
c) RNA (293 Repts) - transferred; synthesizing the RNA probe in progress.

2. Cancer project:Growth Curve: pm 975 Growing:

total cells Day 1 =  $8 \times 10^4$  pfu/ml (New) Day 1 & 5  
 plasma supernatant 3 =  $7.1 \times 10^7$  " 309 is now being  
 " 5 =  $1.3 \times 10^7$  " titrated.  
 " 6 =  $1.1 \times 10^7$  " Day 6 - all  
 " 7 =  $7 \times 10^6$  " all on floating

Growing:

Day 1, d1 1101/1107  
 Day 3, d1 1101/1107

These points  
 are being  
 titrated by  
 stain mixture!

Day 1, d1 309  
 Day 5, d1 309

Growth arrested:

GA 1 DAY = d1 1101/1107

GA 1 Day = d1 309

GA 5 Day = d1 309

Need to call Bruce.

EXHIBIT

C21

3-21-97

Kostya

1) Sequencing of p21 (Nriphen's plasmid, EcoRI-B fragment of pBUG11, EU <sup>12-14 kb</sup> promoter deleted).

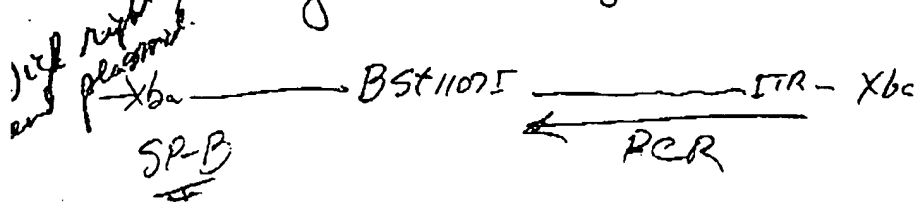
Primers directed to deletion point. <sup>SalH</sup> <sup>SP-C</sup> <sup>SP-B</sup> <sup>CMV</sup>

i) Ad5 sequence is OK ~ 600 bp downstream the deletion.

ii) Upstream the deletion Ad5 sequence 55770 → 55858 is as predicted. Upstream of Ad5 35858 ut there is sequence which is ~~transposon 5~~ <sup>in ITR</sup> *E. coli* Tus according to BLAST.

Probably, ITR is in plasmid but it can not be concluded from this sequence. Moreover, even if it is there, Tus is inserted - 77 bp from the right end of ITR <sup>Tus is at least 500bp or more much larger.</sup> making it not recognizable to virus.

2) It is possible to cut out defective sequence from p82 (large plasmid with SP-B promoter) with Bst1107I and XbaI and put PCR product corresponding to Ad5 right ITR in single cloning.



Nriphen - site in  
SalH promote.  
Review Gold project.

EXHIBIT

C22

Karoly 3/2

Fork project

- I have transduced 12 283 CrmA clones and got partial protection in 7 of them. I will run a Western blot.
- I have tested 15 MCF7 crmA-mIL clones in a Western blot (using a hFosL). All seem to be negative. I will try mixing experiments.
- mFosL viruses: no blue plaque in 2nd plaque purification.

Lower Therapy project

- Growing K138 infected with 100 pfu/cell 11/1/07: no CPE and
- Arrested K138 infected with 100 pfu/cell on 9/5: no CPE and
- Cell proliferation assay (BrdU incorporation  $\rightarrow$  IF) shows that growing K138 is not really growing.

EXHIBIT

C23

Mohan, 3/21/97

1 C.F. Project:

- 1st transfection was done last week
- a) 2<sup>nd</sup> Transfection was done in 293 cells;  
pBHG10 + pAE1sp1A/3.7 SPc Rep78
- b) Northern Blot: for 293 / Rep78 cell line, expressing  
Rep78 Antisense RNA; blot is exposed to film.
- c) checking E1 expression in KB MMTV E1 spinner cells can  
induced by dexamethasone → Samples given to Todorov  
Grow E1/E3 cassette stocks. Start 5 ITR

2 Cancer Project:

Growing 1 day:  $dl 309 = 4 \times 10^4$  pfu/ml  
5<sup>th</sup> day:  $dl 309 = 4 \times 10^6$  pfu/ml

Growth arrested: 1 day  $dl 309 = 1.4 \times 10^5$  pfu/ml  
5<sup>th</sup> day  $dl 309 = 1 \times 10^6$  pfu/ml

Growing day 1 =  $dl 1101/07 = 4 \times 10^4$  pfu/ml

Growth arrested day 1 =  $dl 1101/07 = 1.2 \times 10^3$  pfu/ml

Buy spinners from MC

EXHIBIT

C24

## Growth Curve. HEI 299 cells

Growth Curve				all	
Growing:	pm 975	d1104	d1389	pfu/ml	(100 pfu)
Day 1	$8 \times 10^4$	$4 \times 10^4$	$4 \times 10^4$		
" 3	$7.1 \times 10^7$				Repeat @ 500 PFU
" 5	$1.3 \times 10^7$	$1.4 \times 10^4$	$4 \times 10^6$		Do ~ 3-10A - to identify early onset
" 6	$1.1 \times 10^7$				More CPE data @ 500 PFU
" 7	$7 \times 10^6$				
10					Repl - next week @ WI 38
15		$4 \times 10^4$			
19					
23		$1.3 \times 10^6$			
24					
Growth arrested	Days				
	1	$1.2 \times 10^3$	$1.4 \times 10^5$		
	3				
	5	$1.1 \times 10^3$	$1 \times 10^6$		
	6				
	7				
	10	$1.2 \times 10^5$	$1.4 \times 10^3$		
	14	$3.2 \times 10^5$			
	15				
	19	$1.8 \times 10^5$	$8.4 \times 10^3$		
	23		$5.6 \times 10^3$		

EXHIBIT

C25



Mohan, 4/4/97

1

C.F. Project:

a) 3<sup>rd</sup> Transfection was done; pBHL6 + pAE1sp1a/3.7SPC  
 Next week (8-20 days, should start seeing plaques.  
 (from 1st transfection) On 293 Rep78

b) Northern blot: 293 cells expressing antisense Rep78;  
 RNA from 293 cells itself, gave hybridization  
 signals (bands) with sense and antisense Rep78  
 RNA probe. May be RNA contamination? or  
 unspecific bands?

293 cells transfected again c Rep78 antisense  
 (pCER43 Rep78); when the G418 resistant  
 colonies form, individual colonies will be  
 propagated for the above RNA studies (or PCR).

c) checking expression of EI in KB MMTV EI Spinner cells.

i) KB MMTV EI cells induced with dexamethasone  
 expressed EI B 19 K; for EIA expression the  
 antibody (-Tudor) is not good.

ii) I made a B46 E3 ITR LacZ virus prep from  
 KB MMTV EI Spinner cells (~1.5 liter); so EI is  
 functional in this KB MMTV EI spinner cells.

iii) From 3 liter KB MMTV EI spinner Spun down, 0.15M  
 dexamethasone for 39 hrs gave viral band same as above

2.

Cancer Project:

Experiment 3: HEL 299 growing and growth arrested  
 cells are infected with dl 112/mt; dl 309  
 (500 ppa/cell) = under progress for  
 growth curve experiment

New graph/table for trip to GRI.

EXHIBIT

C26

FasL project

- I did a Western with the 293 CmnA clones, they all express CmnA. I will cotransfect them to make FasL virus.
- Use this cell line to make Ad-FasL vector, perhaps Ad-14.7k and Ad-RID vectors.
- Transfect FasL into cells. 1st exp - got protein.

E3 proteins

- I have isolated plagues from CMV RID and CMV 14.7 transfections and VRID and V 14.7 transfections.  
*Neppen RID  
Pot promoter, R-orientation*
- Expression of RID from the CMV RID construct is ~~not~~ not proven yet.

Cancer Therapy

- I am liking 1101/1107 yields on W138
- There is some CPE in gm875 (100 ph) infected W138 on day 15

Make Ad vector (gene therapy) that expresses CmnA.  
Use for gene therapy to keep transduced cells alive.

Lynda's expression cassette

all promoter, all Ad E3 genes. 1st 293, then PKF-7.  
Do IP

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

C27

4/11/97 Kostya

- 1) Cotransfection of 1101/1107 / EcoRI + p101  
(dl309E3, gp19K mutated) or p111 (dLE3X6a + ADP):  
There are 1 plaque with 101 and 1 plaque with 111  
and no plaques in control dishes. Now amplifying  
these plaques to analyze the genomes.
- 2) Need TK HSV-1 gene from GT1 (if possible, with  
sequence and plasmid map). I'm going to insert  
the TK gene in PaeI site of p101 (in gp19K gene)  
and XbaI site of p111 (~~between~~ upstream from  
ADP insert).
- 3) Failed once to replace Tus sequences with  
ITR in p82 (dLE4 promoter, SP-B promoter insert),  
now repeating the cloning.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

C28

Mohan 4/11/97

Gene therapy Meeting1. C.F. Project:

- a) Three different transfections were done to ~~make~~ construct SPB Rep78 virus. No plaques - yet in <sup>transfection</sup> one transfection (24 days).
- b) Dishes showed CPE like? in ~ 16 days; plaque titrations were done. Started 4-11-97
- c) 293 Rep78, antisense cell line were brought from the freezer; going to repeat the above transfections in Rep78 As. cell line.
- d) primers are ordered for checking AdV Rep78 in 293 cell line.

2. Cancer project: HEL 299 cells, 500 pfu.

- Growing cells showed good CPE at ~ 15 days (4/11/02)
- Growth arrested cells ~ 11<sup>th</sup> days very little CPE?
- dishes are being frozen for growth curve data

3. KB MMTV1:

pBabe puro plasmid is under preparation for Co-transfection with ~~#1~~ plasmid; pCina plasmids.

EXHIBIT

C29

Have p.d. 14.7, cell stock, titer  $1.4 \times 10^7$ 

Keroly 4/11/87

E3 proteins

Do I? of this vector, compare to A15.

Need  
to repeat

- Tested two MPK 14.7 viruses for  $\beta$ -gal-are expresses.
- Tested 3 CMV 14.7 and 4 CMV R10 viruses for  $\beta$ -gal - none of them expresses.
- I have tried to sequence the CMV-R10 construct, did not work. *lysis - point mutations in entire E3 region of A15*
- *Used to do this, CMV promoter.*

Fest project

- I have a <sup>mixing</sup> ~~mixing~~ experiment running with MCF7 - luma - Fest cells, so far it is a may be.

Cancer Therapy

Preliminary titers:

Growing <sup>U138</sup> ~~U138~~ infected with 500 pfu/cell 1101/1107

D1	<	$10^5$
D3	$\approx$	$1 \times 10^5$
D7	$\approx$	$3 \times 10^5$
D10	$\approx$	$2 \times 10^5$
D15	<	$10^5$
D20	$\approx$	$8 \times 10^5$
D25	$\approx$	$1.5 \times 10^6$
D30	$\approx$	$5 \times 10^5$

Quiescent U138 infected with 500 pfu/cell 1101/1107

D 15	<	$10^5$	} <u>no plaques</u> at $10^{-5}$ dilution.
D 20	<	$10^5$	
D 30	<	$10^5$	

Will have ~~U138~~ data on U138 next week.

EXHIBIT

C30



4/18/97 Kostya

- 1) Plaques, resulting from p101 (sp 19K mutated) and p111 (XbaI(E3 + ADP): I have Hirt preps of genomes of these viruses, now analyzing by PCR and restriction digestion.
- 2) I have got clones with T<sub>u</sub>S → ITR substitution. Going to analyze them.
- 3) Starting to construct plasmids with Ad5, pm 760 RID genes under the control of CMV promoter. Same with the 14.7 gene.

Kostya will put E3 transcription unit, containing the pm 794.1 ADP mutations, behind the CMV promoter, for the purpose of inserting into E1 region of BAC11 (or 10).

EXHIBIT

C31

IX only 4/10/54

E3 proteins

- I am going to test more MP6K 14.7 and MP6K R10 viruses. (I am infecting today.)
- I did an IP with MP6K p18k virus, no band on ON exposure.
- I have got the oligos for sequencing the CMV R10 construct, I will do it this weekend.

FasL project

- I am growing up 293 cells and making a CsCl stock of the plasmid (CMV in FasL in left end plasmid)

Cancer therapy projectPreliminary titers WI-38

		500 PFU/cell		100 PFU/cell
	1101/1107	975		308
	A	G	A	G
D1		$1 \times 10^5$	$1 \times 10^5$	
D3		$5 \times 10^5$		$3 \times 10^6$
D5			$1.5 \times 10^5$	$1 \times 10^7$
D7		$3 \times 10^5$		
D10		$4 \times 10^5$	$1 \times 10^6$	$3 \times 10^7$
D15	$< 10^5$	$< 10^5$	$2 \times 10^5$	
D20	$< 10^5$	$8 \times 10^5$	$8 \times 10^5$	
D25		$2.5 \times 10^6$	$7 \times 10^5$	
D30	$< 10^5$	$7 \times 10^5$		

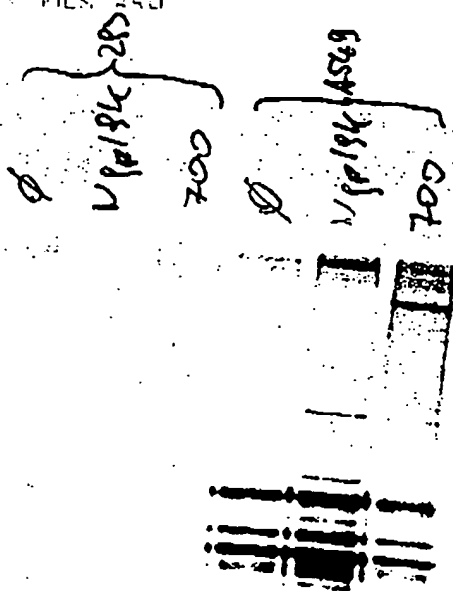
EXHIBIT

C32

KODAK SAFETY FILM 351

KODAK SAFETY FILM 351

4/18



Could see by IF, beginning at 2 days pi

EXHIBIT

C33



Mohan, 4/18/97

1. C.F. Project:

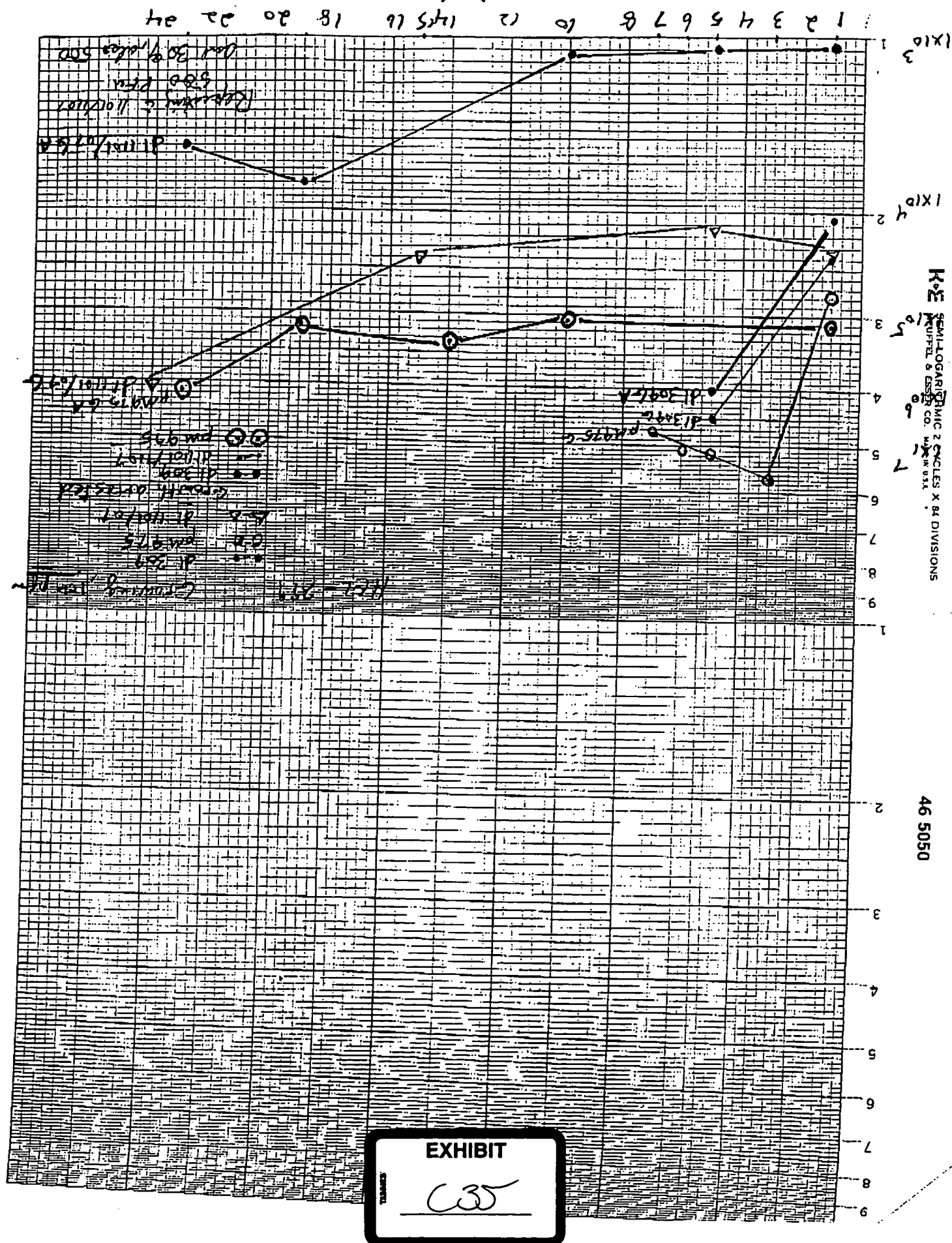
- a) No plaques in 293 cells co-transfected with pBHG10 and pAE1SpA/3.7 SpC Rep78. (~ 30 days and 24 days); 3<sup>rd</sup> transfections (~ 18<sup>th</sup> days) dishes are stained; waiting for plaque plaques.
- b) Cells showed CPE like effect (pBHG10 + pAE1/SpC Rep78) were titrated and not stained yet. But even undiluted dish did not show CPE.
- c) RT-PCR showed no Rep78 DNA fragment in 293 and 293 Rep78 AS cell lines.
- d) Spinner KB MTV E1 cells - Virus IR LacZ is ~~under~~ in spinner; Karl virus is going next.
- e) pBabe puro plasmid is made in large quantities; for transfecting the KB E1 cell line with Et and Cmx A plasmid separately.

2. Cancer project:- HEC 299 cells; 500 pfu  
- Growing cells were dead at ~ 21 days.  
- Growth arrested cells 18<sup>th</sup> day. On 21<sup>st</sup> day cells will be frozen for Growth Curve analysis.

3. preparations: plasmid BHG10, BHGE3, pAE1/SpC Rep78; will be for transfection. C34 purified.  
293 Rep78 AS cell line will be co-transfected with  
BHG10 + pAE1 SpA/3.7 SpC Rep78 plasmid  
BHGE3 + pAE1 SpA/3.7 SpC Rep78 plasmid  
KAS DNA + pAE1 SpA/3.7 SpC Rep78 plasmid

EXHIBIT

C34



4/25/97 Kostya

- 1) I have got the virus 1101/1107 with gp19k mutated (restriction digestions and PCR).
- 2) Now preparing Hirt's from 4 plaques which should be 1101/1107 E1; Xba E3 deletion, ADP insert.
- 3) Failed to substitute T<sub>us</sub> → ITR. Going to do it in 2 clonings but without partial digestions.
- 4) Sequencing shown that ITR is OK in Lyda's plasmid; could not sequence through ITR-ITR junction in pBAC10, 11; I have repeated the sequencing with different PCR protocol, now waiting for results.
- 5) I have checked with Ann, she doesn't have plasmids with rec 700 or pm 734.1 which would be suitable to cut out SrfI - NdeI fragment corresponding to whole E3, but she does have the viruses. Now going to prepare rec 700 and pm 734.1 DNA to insert SrfI - NdeI fragment to pCDNA3 / EcoRV site.
- 6) The virus 544 (1101/1107 E1; Lyda's deletion of E3 ~~(1101/1107 E1)~~, 12.5 k ATG mutated, d127984-28133 d128390-30883) + ADP insert into PaeI site in place of second deletion. → I gave the virus to Shari to grow up CseL prep.

Need TK gene from ATI.

EXHIBIT

C36



Keroh 4/25/97

E3 proteins

- I have tested more MP6K 14.7 and E1D viruses. The ones that stain for p-Gal show some staining in 1F for 14.7 or 14.5. The E1D p staining shows the same pattern as the previously made ~~Ad~~ Ad E1D viruses. In addition to this, the cells that stain are dead ~~for~~ (carryover from 293?). The 14.7 staining looks normal. I am growing up more of the positive clones.

- FesL project

- I have two transfections going in 293 Com A.

Cancer Therapy project

- I have infected MCF-7 with 01/07, 975 and 308. Will do the same with A549 and 293 and take a growth curve.
- I am infecting W138 for the missing timepoints.
- I am titrating the remaining samples.

EXHIBIT

C37



1101/1107

175

308

A

G

A

G

A

G

D1

 $1 \times 10^5$  $1 \times 10^5$  $4 \times 10^5$  $3 \times 10^6$  $1 \times 10^5$ 

D3

 $5 \times 10^5$  $1.5 \times 10^5$  $3 \times 10^5$  $1 \times 10^7$  $2 \times 10^7$ 

D5

 $3 \times 10^5$  $1 \times 10^6$  $1.4 \times 10^7$  $6 \times 10^7$ 

D7

 $4 \times 10^5$  $2 \times 10^5$  $2 \times 10^7$ 

D10

 $1 \times 10^5$  $8 \times 10^5$  $1 \times 10^6$ 

D15

 $2.3 \times 10^6$  $7 \times 10^5$  $1 \times 10^6$ 

D20

 $7 \times 10^5$  $1 \times 10^6$ 

D25

 $< 10^5$  $2 \times 10^5$  $< 10^5$ 

D30

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

C38

She 11 Chart 2

Approximate titers in WI38 4/25/97

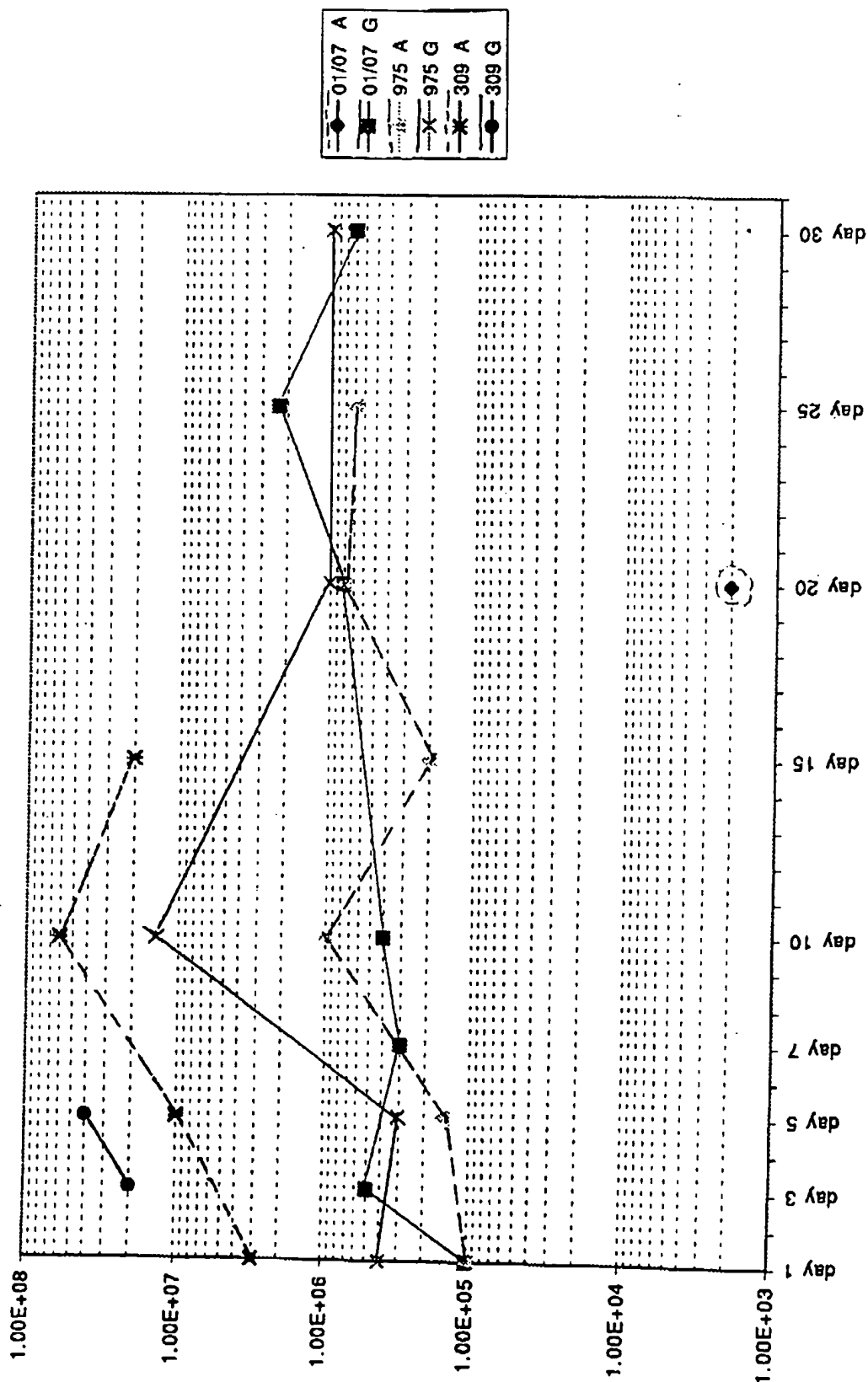


EXHIBIT  
C39

Nohar, 4/25/97

1 C.F. Project:

- a) cells showed CPE like effect (co-transfected pAE1SpA/3.7SPC Rep78 and pBAGLO) were titrated. No plaques yet ~15<sup>th</sup> day. But when 293 cells infected with CPE, CPE like started - look Ad CPE typical.
- b) In 293 Rep78 AS - cell line, co-transfections of pAE1SpA/3.7SPC Rep78 and pBAGLO were done; pAE1SpA/3.7SPC Rep78 and pBAGLO E3 plasmids.
- c) Virus Ct 19.7 are growing in KB MMTV E1 Spine cells.
- d) GAPDH (Glyceraldehyde 3Phosphate dehydrogenase) primers - ordered.
- e) pBAGLO, pBAGLO E3 under preparation for further transfections.
- f) I am going to isolate A17001 DNA for transfection → Virus Rep78.

2 Cancer Project: HEC 299 cells

- Growing and Growth arrested cells are frozen for growth Curve analysis.

EXHIBIT

C40

9/2/97 Kostya

- 1) Terry's gp19K plasmids. pCR11gp19K Ad5 turned out to be pCR11gp19K Ad2. pC1gp19K contains gp19K Ad2 in correct orientation. (Karol - Transfection - IP).
- 2) Cloning of the complete E3 under CMV promoter:
  - i) There was E3 inton in Horowitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
  - ii) Started to extract pc700 and pm734.1 (m1m41) genomes to clone E3 to pCDNAB (or pC1?).
- 3) Cloning experiments to replace ITR in SP-B ↔ E4 promoter plasmid are in progress.
- 4) Got the virus 1101/1107 E1 <sup>grows much better than dl7001</sup> dlE3 Xba + ADP - Checked with restriction digestion and PCR. Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + ADP; dl309 gp19K untagged; dlE3 Xba + ADP). Using dl309; 1101/1107; pm734.1 (m1m41); 7001 as controls. Cell lines - 293 and A549. Going to start with IP to check for ADP expression. I'm really ready to put TK into 2 last recombinants E3, need TK plasmid and TK sequence from GT1.  
Need TK from GT1!

EXHIBIT

C41



Karsky 5/2/87

ES proteins

- I am growing up NP6k 14.7 #5 stained best IF.
- I did an IP with transfected p13k and 14.7 constructs (in 293 cells). All but pCBK3 14.7 expressed, all comparable to virus injection. (Labeling 38 h post TF and 18 h p.i. for 3h)
- I did an IF with the same transfections (in MCF7 cells) it did not work, I am repeating it.

lacZ pGK-14.7  
Protein got moderate band.

Fas L project

- I am waiting for plaques in 293-Crims cells.  
Have one plaque after 10 days.

Cancer Therapy project

I have infected	MCF7	01/07 500 ph	975 100 ph	309 100 ph
	A548	500 ph	500 ph	500 ph
	293	500 ph	100 ph	100 ph

293 is rounded up 1 day p.i.  
A548 2 days p.i.  
MCF7 5 days p.i.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

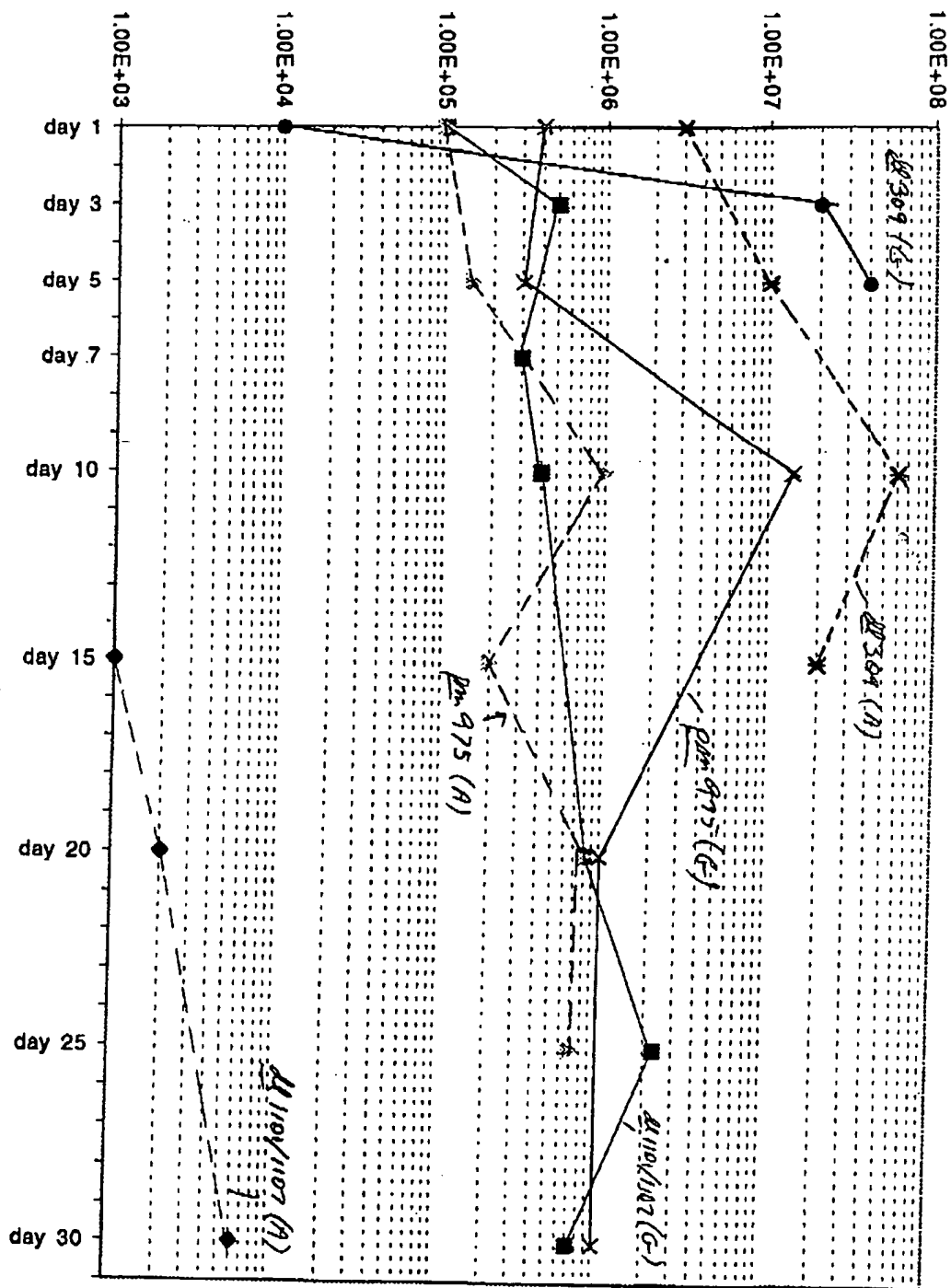


EXHIBIT

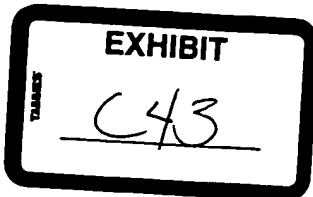
C42

Sheet1 Chart 2

Approximate titers in W138



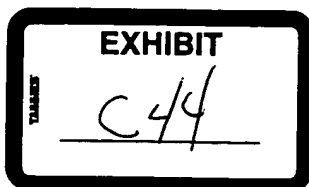
—◆—	01/07 A
—■—	01/07 G
—x—	975 A
—*—	975 G
—●—	308 A
—○—	308 G



5/2/97 Kostya

- 1) Terry's gp19K plasmids. pCR11gp19K Ad5 turned out to be pCR11gp19K Ad2. pC19gp19K contains gp19K Ad2 in correct orientation. (Karol - Transfection - IP).
- 2) Cloning of the complete E3 under CMV promoter:
  - i) There was E3 inton in Horowitz's virus, their construct starts ~200 bp after E3 transcription start in Ad2 E3.
  - ii) Started to extract Rec 700 and pm 734.1 (m1m41, genomes to clone E3 to pCDNA3 (or pCI?).
- 3) Cloning experiments to replace ITR in SP-B ↔ E4 promoter plasmid are in progress.
- 4) Got the virus 1101/1107 E1, <sup>grows much better than dl7001</sup> dlE3 X68 + ADP. Checked with restriction digestion and PCR. Recently started experiment (plaque assay) to compare growth properties of 3 recombinants (Lynda's deletion E3 + ADP; dl309 gp19K unmutated; dlE3 X68 + ADP). Using dl309; 1101/1107; pm 734.1 (m1m41); 7001 as controls. Cell lines - 293 and A549. Going to start with IP to check for ADP expression. I'm really ready to put TK into 2 best recombinants E3, need TK plasmid and TK sequence from GT1. Need TK from GT1!

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1. C.F. Project:

- a) BHCE3172 LacZ virus gave moderate band in CSCI banding. But the  $\beta$  gal activity/ml is  $\sim 1.4 \times 10^9$  which is same as previous 172 LacZ preparation. Second set is being titrated to compare the pfu/Lfu/ml.

L J may use Centricon 100 (M.Wt cut off 100,000) to concentrate the viral stocks for one CSCI spinning which will increase the pfu. Dialysis of stock 172 LacZ virus will increase the volume and it is hard to spin in one tube.

- b) Co-transfection of pELSp1A/3.7 sec Rep78 & pB2610; pB26E3 in 293 Rep78 antisense cell line will be stained next week.

2. E3 project:-

- a) Virus C+14.7 (pGKII 14.7 + p gluc) gave moderate band in CSCI spinning. Viral titrations are in progress.

- b) Going to make another virus 14.7 LacZ (pGK 14.7 in E3; LacZ in E1) preparation. - infection to day

3. Cancer Project:-

Growing and growth arrested virus infected PLE 293 cells ~~extract~~ extract will be titrated for growth curve next week.

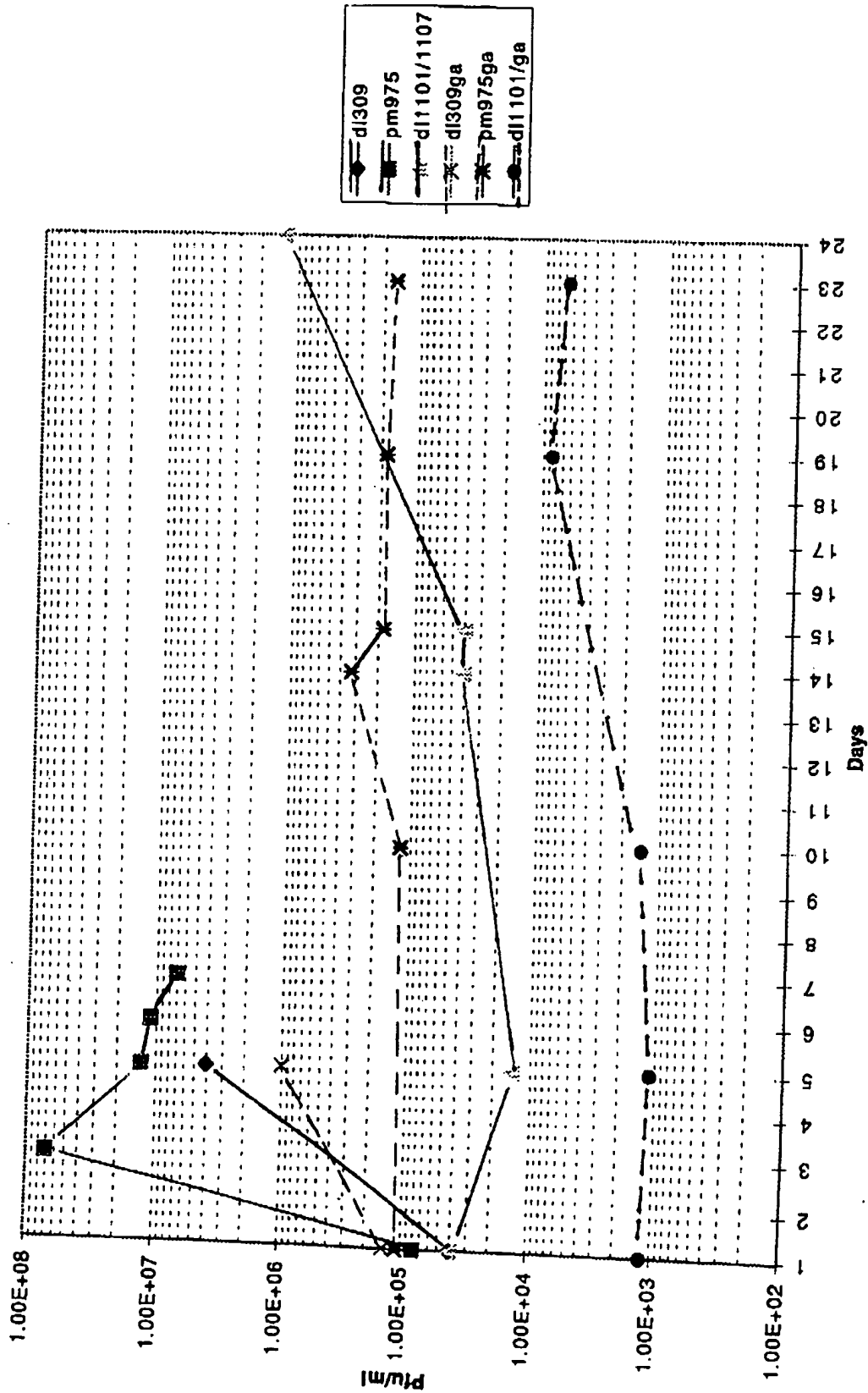
22110/1107 at 500 PFU/cell  
22309 " " "

EXHIBIT

C45

Sheet1 Chart 3

Growth curve 3



EXHIBIT

C46

Kostya

- i) Results of cotransfection 1101/1107 / EcoRI + p54 (dlE3 + ADP) → 4 more plaques were analyzed. 3 of them contain recombinant genomes.
- ii) Subcloning of dl 309 E3 with gp19K mutated and dlE3 Xba + ADP into Linde's plasmid is in progress. When the plasmid with 19K\* is made I'm going to insert HSV-1 TK into PacI site created in gp19K gene.
- .) i) No plaques with p820 (pB611 E4 promoter → CMV promoter). No plaques with pB611 as a control.
  - ii) 4 more plaques resulting 1101/1107 / EcoRI + p82 (E4 → SP-B promoter) were analyzed. All contain wt genomes.
  - iii) In 2 repetitions of cotransfection 1101/1107 with p82 (E4 → SP-B promoter) no plaques neither in control nor in experiment. 2nd experiment was done on Mohan's 293/CMV-TTF cells. Not repeating the cotransfection.
  - iv) Sequencing of Nippen's plasmid p21 (E4 promoter dl) is in progress to prove that right Ad ITR is intact in the plasmid.
- i) Growing initial stocks of the recombinant virus with dlE3 + ADP.

EXHIBIT

C47

Gene Therapy Group

Koroly 5/8/97

E3 proteins

- I am growing up MP6k 14.7 #5
  - I did an IF with transfected p13k and 14.7 constructs in MCF7 cells, all of them but pCDNA3 14.7 expressed well.
- Virus - MP6k, p13k and pGlu 14.7 were negative after 20h.  
Nelson is doing a longer infection assay

Fast project

- I have 4 plaques. They appeared 10-11 days after transfection with BTHG11 in 293 Crm A cells. Will pick one plaque (large) today.

Cancer Therapy project.

293, MCF7, last time mistake: MCF7  
Repeat: 293 A49.

I am repeating A548 infections and some U138 infections.

CPE in U138 cells

A = 0.2% serum FCS  
G = 5% FCS

01/07 A

01/07 G

No CPE in 1101/1107, either in

no CPE

"atypical CPE"  
starving around  
day 10

500 pfu

The "growing" don't  
really grow.

growing or  
more growing.  
But, have fewer cells  
in the growing than  
non-growing

975 G

No real

975 A

D2 +/-

diff. between

D4 +/-

D3 +/-

G and A

D5 +

D4 +

for p975

D6 ++

D5 ++

500 PFU

D7 +++

D6 ++

B08 A

308 G

D5 +

D3 +/-

D6 +

D4 +

D7 +

D5 ++

D7 +

D1 +/-

D7 +

500 pfu

For 100 PFU, growing was same as 500 PFU  
But, arrested did not show CPE  
until ~ 15 days. Same as HEL-299.

100 pfu

also pCI

Buy pCDNA3.1. Clone 9p19, 14.7, RLD  
D12-14.7 D12-14.7

EXHIBIT

C48

2 photograph  
as done with  
0 PFU.

last week  
2L plaques  
A - CAT test for  
free ERF  
'98, HEL-299

missing recap  
if cells taken

5/9/97 Kostya

- 1) Construction of the plasmids with whole E3 (rec 700, pm 734.1) is in progress.
- 2) I have constructed the plasmid with E4 $\leftrightarrow$ SP-8 where T<sub>u</sub>S $\leftrightarrow$ ITR, sequenced the plasmid, now waiting for sequencing results to make the large version of the plasmid for cotransfection.
- 3) For 101-1 (dl 3.9 E3, gp 19K untested) plaques are appearing the same day as for 309. (day 4)  
For 111-1 (E3 XbaI dl + ADP) plaques appeared 1 day before dl 309. (day 3)

① KD-1. 1101/1107 in Lynda's E3 background + ADP  
No promoter for ADP.

Shari harvested day 2 pi, got band, well t<sub>u</sub>ka.  
Cells were beginning to die. Shari will titer.

② KD-2 1101/1107, 309 E3, gp 19K untested.  
Plaques appearing same day as 309.

③ KD-3 1101/1107, XbaI deletion (dl 327 deletion),  
ADP without promoter, but in-frame c. 6.7K.  
Got plaques 1 day before dl 309 (ix on day 3).  
Got this <sup>one</sup> plaque 4 days posttransfection, 5mm wide.  
Got other 3 plaques 7 days posttransfect  
DNA screened - are recombinant.

Future - could put ADP under control of SPB promoter.  
First, concentrate on what we have.

infect pAD2-ADP with 29.3 cells





Mohan, 5/9/97

1. C.F. Project:

- a) Virus BM6 E3 ITR LacZ gene  $1.4 \times 10^9$  Lpfu/ml (pre-assay)  
 Plaque assay gave  $9 \times 10^8$  pfu/ml (10th day)  
 I received Centricon 50 concentrator from Millipore as  
 samples - going to concentrate the ITR LacZ virus.
- b) So far no plaques for constructing Rep78 virus.
- c) GAPDH - RT-PCR in progress.
- d) Going to transform d1200 DNA (Lat digested, with  
 pSP Rep78 plasmid).

2. E3 project:

- Radical conversion, left-to-right orientation*
- a) Virus C+14.7 (~~p<sub>pol</sub>~~ <sup>pol II</sup> 14.7 + a gene) has been  
 Titrated. No plaques yet - 10th day.
- b) CPE stock for 14.7 LacZ (~~p<sub>pol</sub>~~ <sup>pol II</sup> 14.7 + LacZ) virus  
 has been made. Going to titrate (5ml final)
- c) A549 cells are infected @ 1:700, GP19K and  
 C+14.7 virus for I.F. (2 days & 4 days)  
 Hard 2 days - negative already infected.

3. Cancer project:- HEL 299 cells

Growing, growth arrested, all 11/01/07 (500k)  
 infected - CPE stocks are Titrated for growth curve  
 Will do 309 in coming week

HEL-299 100, 500 PFU Graph: days p.i. vs CPE - will give me.

EXHIBIT

CSO

5/23/97 Kostya

1) E3 project:

Cloning of E3 from rec 700 and pm 734.1  
in pBSSK (+) - got bacterial colonies, analyzing  
then reclone into pCD, then make virus.

2) Cancer therapy project:

- i) Made a large plasmid (Bam → end of genome)  
with E4 → SPB promoter and ITR repaired,  
now making Cae prep. of the plasmid to transfect.

Next week make virus.

- ii) Plaque development assay for E3 ADP viruses:

a) 293 cells (day 18)

Negative control:

7001 - plaques just started to appear (day 18).

734.1 - plaques appeared day 14-15

Positive control:

309 - day 4-5

1101/1107 - day 4-5

Recombinant viruses:

KD1 (544, LKH d1 E3 + ADP) - day 5-6, plaques smaller  
than wt.

KD2 (101-1, d1 309, gp 19K \*) - day 4-5, large plaques.

KD3 (111-1, d1 327 + ADP) - day 4-5, large plaques.

b) A549 cells (day 8)

Same day or a little faster than wt.  
Perhaps a little larger.

positive  
control

309 - day 4-5 (large)

1101/1107 - day 8 (small)

negative  
control

734.1; 7001 - no plaques yet.

recs.

KD1 - didn't make

KD2 - day 5-6, large, smaller than wt. (309)

KD3 - day 5-6, smaller than KD2.

EXHIBIT

C51

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



Mohan, 5/23/97

1. C.F. Project:

- a) Virus BHGE31TR LacZ was concentrated using Centricon filter columns. Lfu is  $\sim 3.86 \times 10^{10}$  /ml (ul/ml)
- b) d1700 viral DNA digested with ClaI and pAE1sp1A/spcRep were co-transfected.
- c) 293 TTF cell lines were transfected with pAE1/spcRep. I.F. stained for Rep78. Most of cells transfected with pAE1/spcRep78 are washed out. Few cells (about 2) showed nuclear staining. But not clear.
- d) 293 L Rep78 antisense cell lines showed GAPDH-PCR fragment not the Rep78 fragment. So, no LAV2 contamination.

2. E3 Project:

- a) NPGK LacZ 14.7 (CPE stocks)  $\text{gand} = 3 \times 10^8$  Lfu/ml in 293 cell but in A549  $= 2.3 \times 10^8$  Lfu/ml  $\sim 10$  fold more in A549 cells than 293 cells

- b) I.F. = A549 cells: TPO - 21 hrs (21 hrs) + + + for 14.7  
 $\sim 50$  pfu/cell NPGK 14.7 - 1 day -  
 2 day -  
 4 day + + + +  
 (Bacteriophage) - 4 day + + (cytoplasmic staining)  
 GP19K = Background

Virus 14.7 expresses 14.7  $\sim 4$  days post infection at wt. level.

Ct 14.7, at 4th day expresses lesser. Mainly cytoplasmic staining and around the nucleus.

Ct 14.7 when titrated  $10^7, 10^8, 10^9$  no plaques. - Titer may be less. So, -IF- may be less.

EXHIBIT

CS2

3 Cancer project: HZL 299 cells 500 pfu/cell

Growing

d1309 d1104/07

3 hrs	$9 \times 10^3$	$2 \times 10^4$
1 day	$1 \times 10^4$	$4.2 \times 10^4$
4 "	$1.6 \times 10^4$	$1.2 \times 10^5$
6 "	$2.3 \times 10^4$	
8 "	$9 \times 10^4$	$1.4 \times 10^5$
10 "		$7.1 \times 10^5$
15 "		$2.2 \times 10^6$
21 "		$4.6 \times 10^6$

Growth arrested

3 hrs	$-4 \times 10^4$
1 day	$4.4 \times 10^4$
4 "	$4 \times 10^4$
8 "	$5 \times 10^4$
10 "	$-1 \times 10^4$
15 "	$-1 \times 10^4$
21	$-2 \times 10^4$

EXHIBIT

C53

Kendy 5/23/97

E3 proteins

- I am doing an IP with NPGK 14.7 and Ct 14.7
- I am growing up lac-Pol II p19K
- I am cloning the MT2 expression cassette in pC12pac (hyuda changed the BamHI and BpII sites to pacI in pC1)

FesL project

- I have isolated 4 plaques <sup>Could be WT.</sup> They grow very fast (kill 293 ON, show CPE in A549 ON).
- I have infected MCF7 and MCF7ermA cells, it shows CPE ON in both.
- I have tried to PCR out FesL from the virus, it failed. Positive control (plasmid) was OK.
- The virus expresses p-Gal
- I am trying to show the protein in IF.

Cancer therapy project

- I am going to hit yields from A549, MCF-7 and 293 next week.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

C54

5/30/97 Kostya

1) E3 project :

Made the plasmid with E3 from pm 739.1 (u1/u41).  
(ADD<sup>-</sup>) - Sfi1-Xba1 fragment in pBlueScript. Now cloning  
is in progress to put it into pCDNA3.1zeo(+)-new  
plasmid with CMV promoter. Now just started.

2) Cancer therapy project :

i) Made cotransfection of "01/1107" / EcoRI with <sup>Bam → mp100</sup> p152 <sup>SPB</sup> <sup>in</sup> <sup>Est</sup>  
(new version of large plasmid Bam - ~~red~~ with  
SPB ↔ E4 promoter substitution). to 293 and promote  
293-TTC cells, cotransfected pCMV/TTC as well. <sup>Hoo ITR</sup>  
(made by Mohan)

ii) Plaque development assay for E3 ADP viruses :

293 cells - see graph.

AS249 cells - in progress.

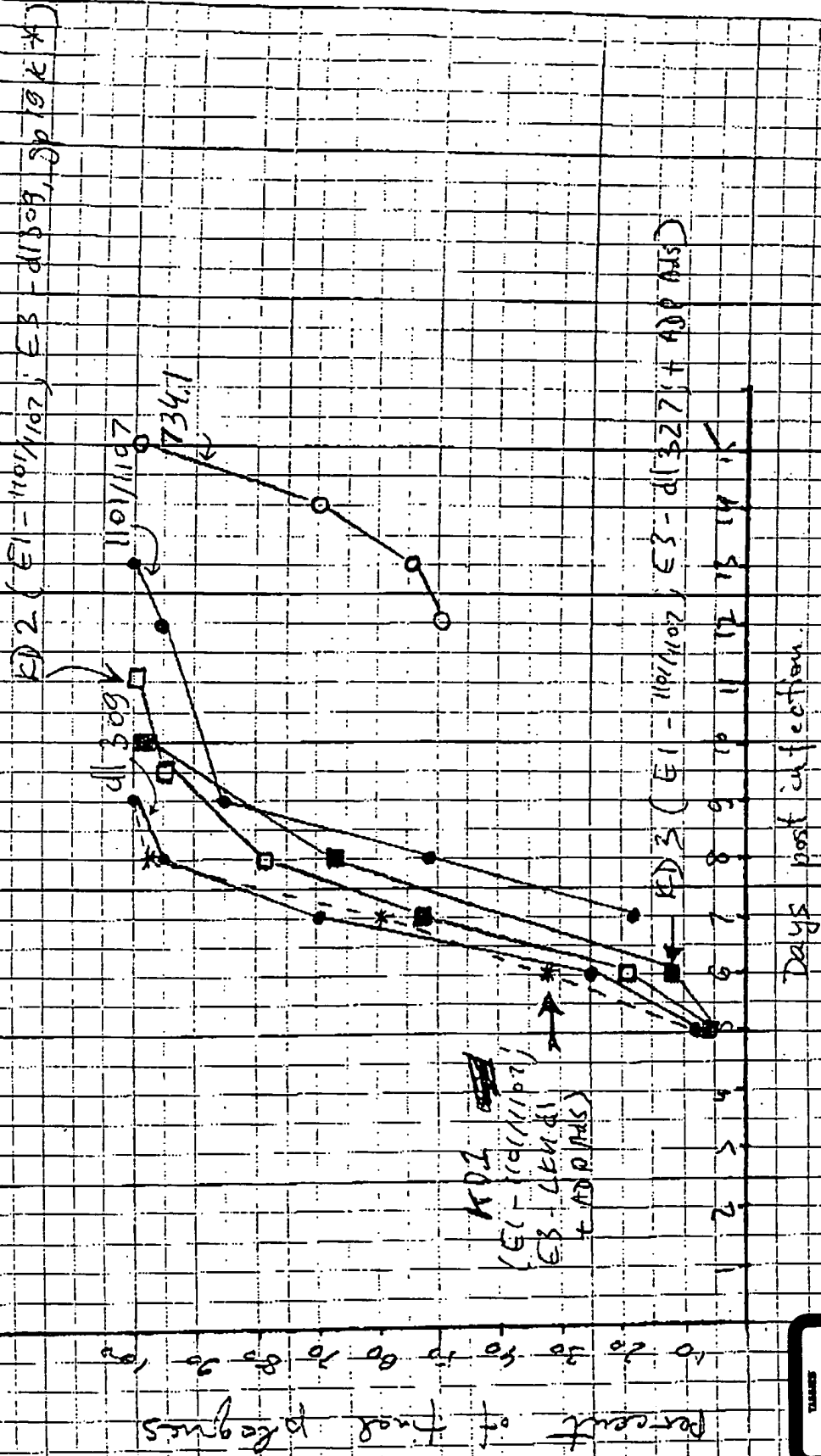
iii) Starting IP with ADP sera to check for  
ADP expression in the viruses direct.

EXHIBIT

CSS

Kostyga

Plaque development assay, AS49 cells



EXHIBIT

957

Korohy 5/30/87

E3 proteins

- I did an IP with NPGK 14.7B, the result is not conclusive.
- I am repeating the MTZ cloning.

Fact project

- The tested four plaques were not expressing  $\beta$ -Gal. I found two blue cells, I don't know where they came from.
- I have 3 new plaques, they came at the same time as lacZ controls.

Crm A

I am cloning CMV-CrmA in the left end phasmid

Cancer therapy

- I am taking the time course in A549, MCF-7 and 283 cells. Started yesterday.

E2-Gal

I am waiting for plaques  
No plaques on lacZ control yet either.

In Chip's GAL4-TAD 283 cell line

(24 and 8 days).

BAG1, E2A promoter substituted  
c Gal4/TATA.

lip- give Ad2 x Ad5 combo. in pCI, to Karl. Make Ad vector  $\pm$  lacZ  
- Give R5KTA-14.7K, pCI-14.7K, pMT2-14.7K to Nolan. Make Ad vector  $\pm$  lacZ  
style. Will handle 9P19K/CMV, Ad2 version.

EXHIBIT

C57

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



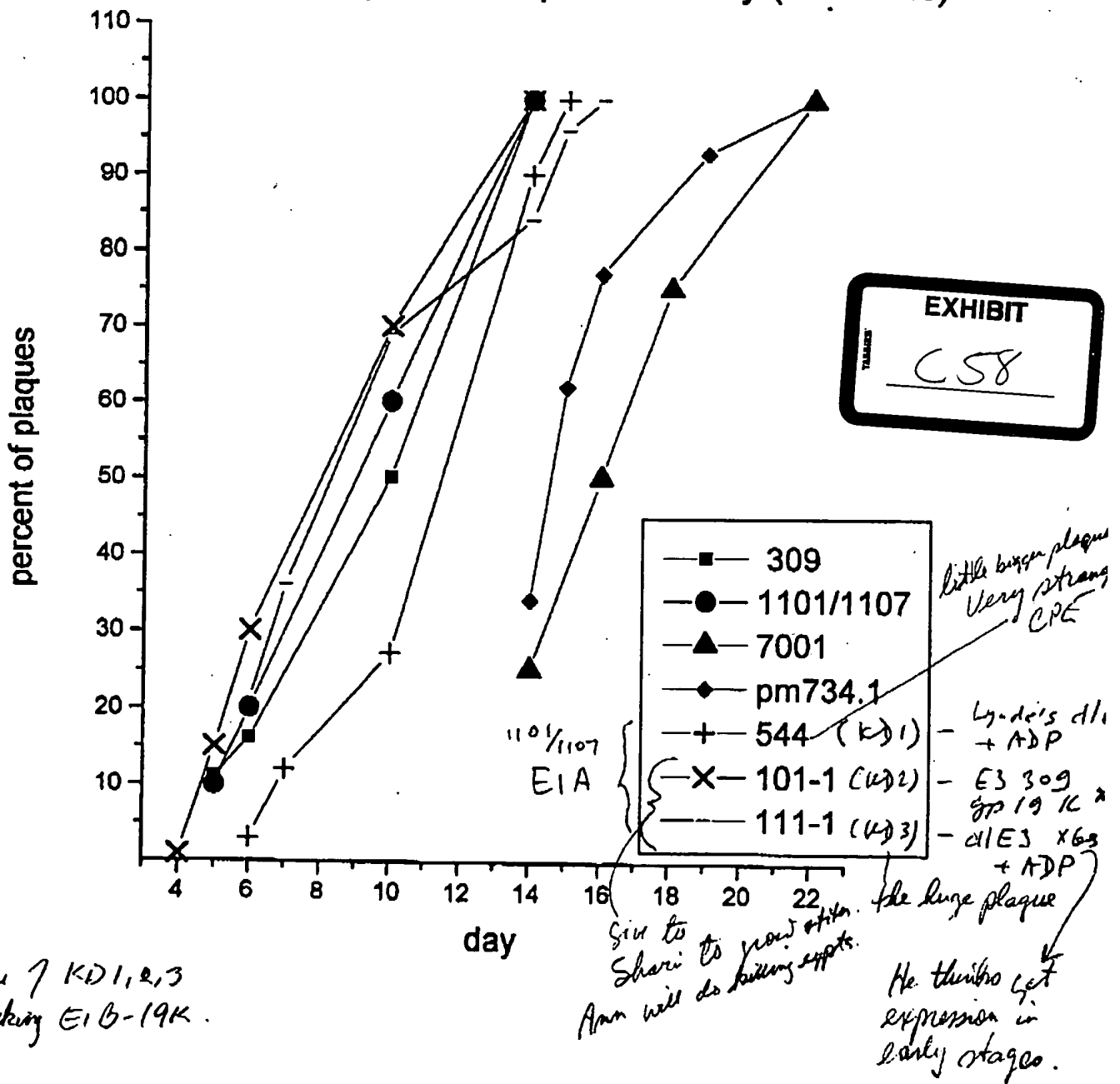
EXHIBIT

C

SERIAL



# Plaque development assay (293 cells)



As219 expt - now at 14 days. Look like 293, but 309 is a bit faster.  
 20 1101/1107 mutant does show plaques on 293.

Mohan 5/30/97

4. C.F. Project:

- a) 293 cells (Rep78) transfected by d17001 DNA (2<sup>nd</sup> started showing plaques (~40/60mm dish) ~ 15 days  
 cells co-transfected with pAE1 Sp1A/37 SPC Rep78 and d17001 + ClaI digested viral DNA  
 no plaques yet. (15th day)  
 cells transfected by d17001 + ClaI - no plaques -  
 (I am going to several transfections using d17001 + ClaI digested DNA)

- b) MCF-7 ~~cells~~ cells were transfected - as following plasmids for I.F. for Rep78  
 1) pCDNA3 Rep78 (I.F. will show Rep78 expression)  
 2) pCMV TTF  
 3) pCMV TTF + pAE1 Sp1A/3.7 SPC Rep78  
 4) pAE1 Sp1A/37 SPC

- c) Primers for Rep78 antisense → ordered.

2. EB project:

- a) viruses C+14.7 (Pacina promoter), 14.7 LacZ  
 (p01 2 14.7)  
 did not show plaques at 10<sup>-7</sup> dilution.

particles/OD; for wt Ad 9.04 × 10<sup>13</sup> / ml - STD.

		1	2	pfu	particles/OD - OPB
Cell	r700	0.174	0.167	9.88 × 10 <sup>10</sup>	~ 1.54 × 10 <sup>12</sup>
	C+14.7	0.232	0.217	~	~ 1.9 × 10 <sup>12</sup>
CPE 50% - OPGK 14.7		0.869	0.861	3 × 10 <sup>8</sup> - 293	---
(medium is interfering)				2 × 10 <sup>8</sup> - 293	---

→ Going to repeat with more dilutions.

- b) After C+14.7 and 14.7 LacZ pfu → I.F.

EXHIBIT

C59

3. Cancer project: HEL 299 cells; 500 pfu/cell

dl309 growth arrested cells → stained  
→ plaques started appearing. Count ~ 2 day

EXHIBIT

C60

Sheet1 Chart 6

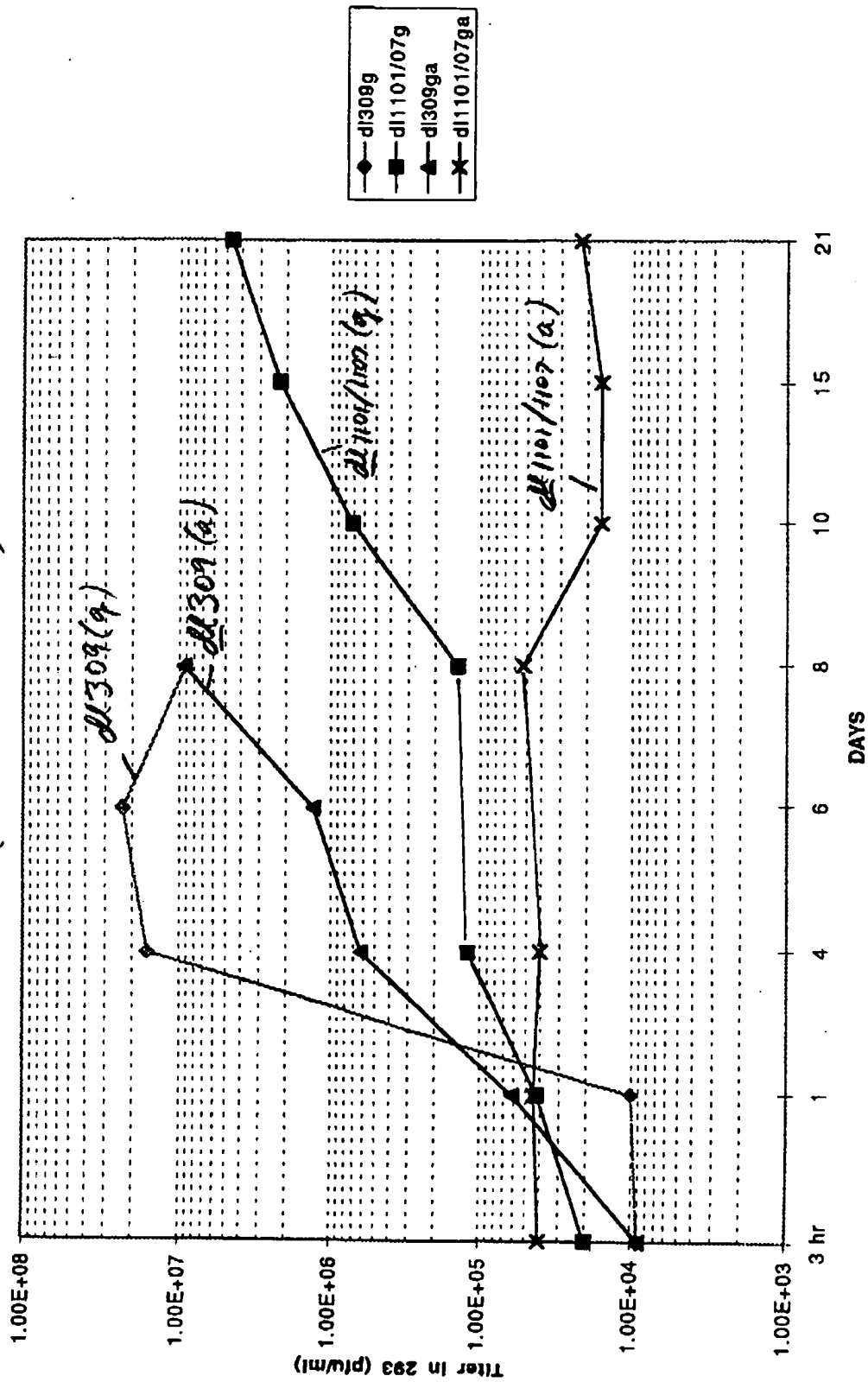
Growth curve 500 pfu/cell  
(HEp 299 cells)

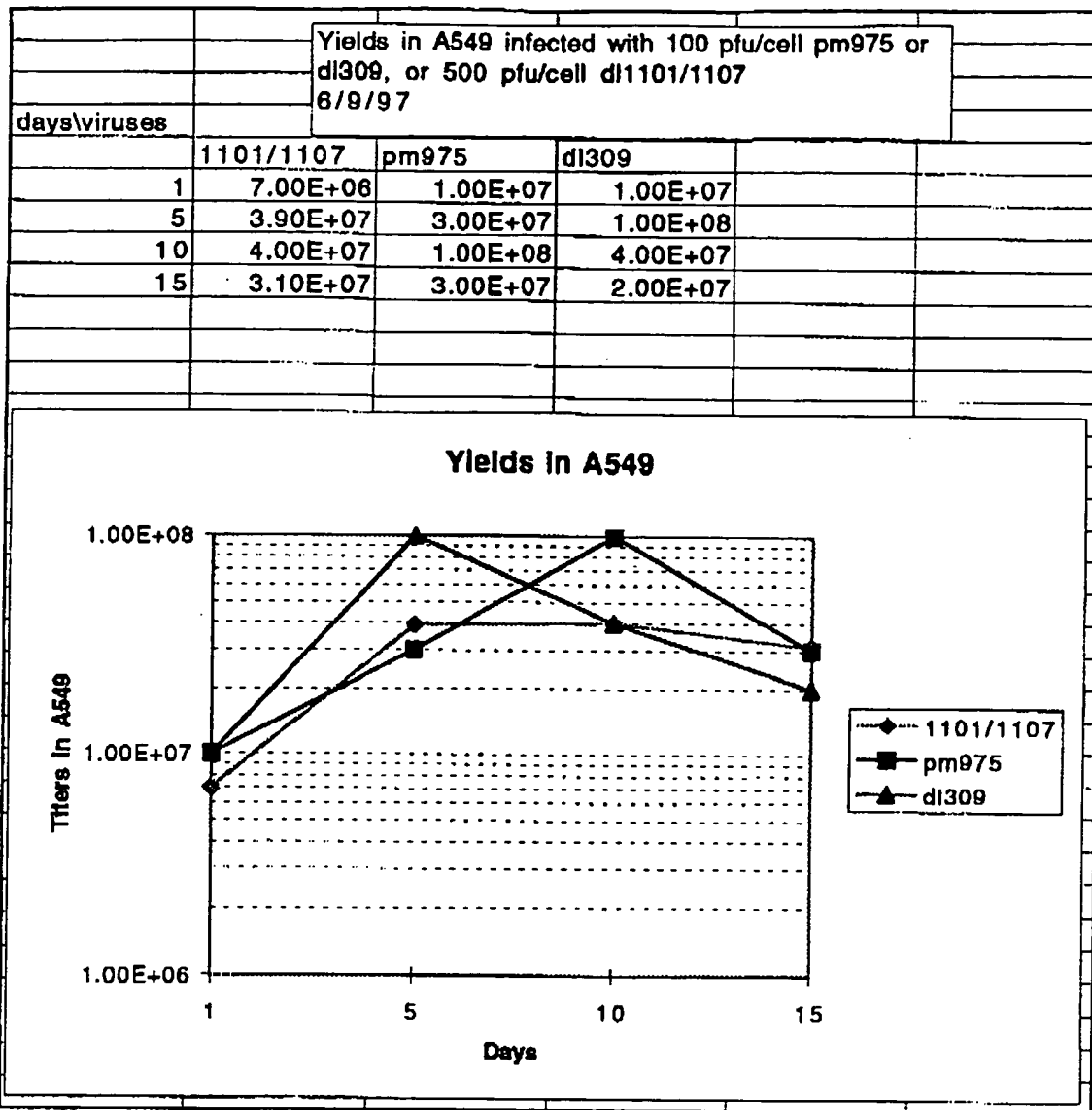
Figure 1.

Page 1

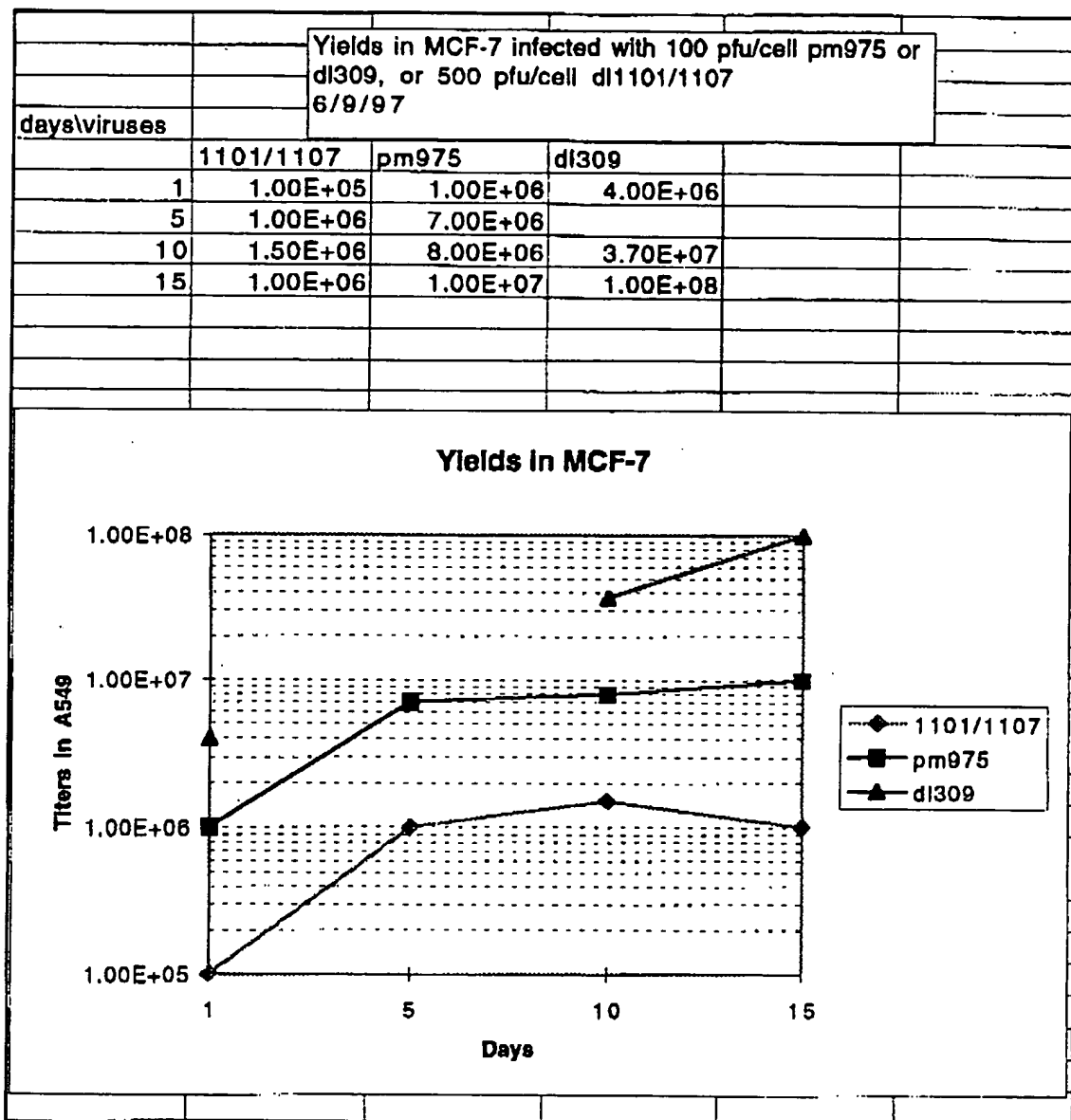
EXHIBIT

C61

Sh et1

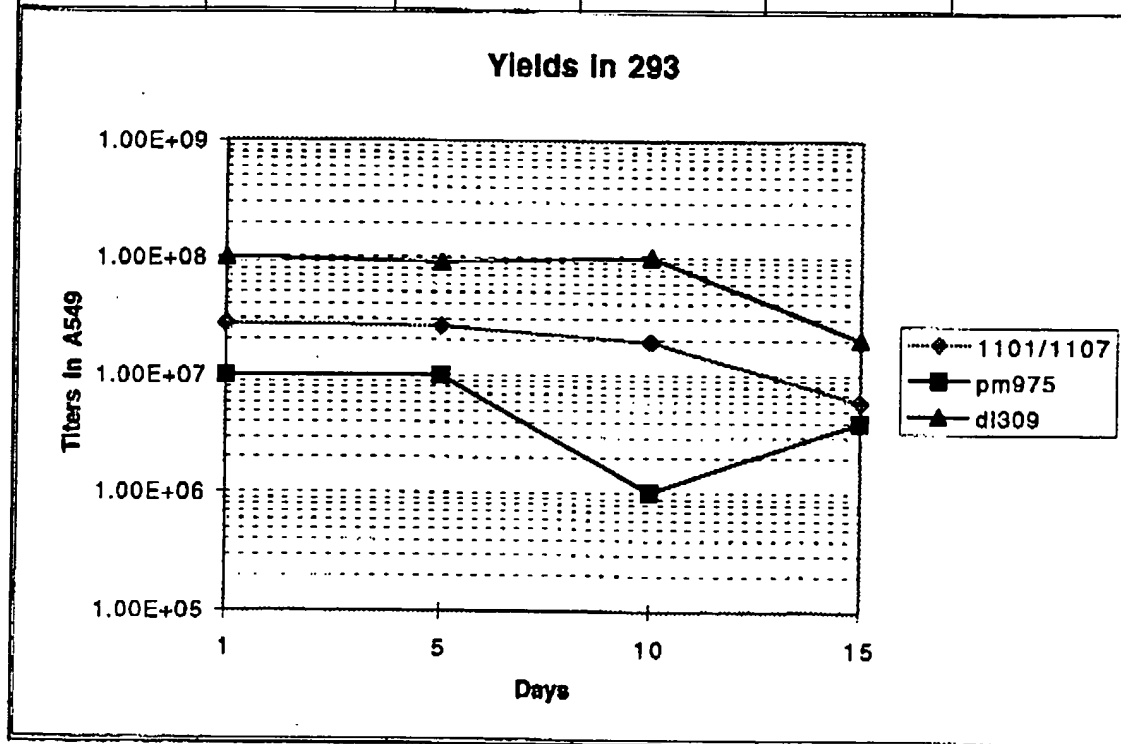


## Sheet1



## Sheet1

		Yields in 293 infected with 100 pfu/cell pm975 or dl309, or 500 pfu/c ll dl1101/1107		
		6/9/97		
days\viruses				
	1101/1107	pm975	dl309	
1	2.70E+07	1.00E+07	1.00E+08	
5	2.80E+07	1.00E+07	9.00E+07	
10	1.90E+07	1.00E+06	1.00E+08	
15	6.00E+06	4.00E+06	2.00E+07	



6/13/97 NOS 1/2

1) E3 project

Made the plasmid with E3 (SrfI-NdeI) from pm784.1 (ADP-) in pCDNA3.1zeo(+). Gave maxiprep to Karol to check for E3 proteins.

2) Cancer therapy project

i) There are no plaques yet in dishes with p152 (E4-SPB) transfection (2 weeks).

ii) Plaque development assay for E3 ADP viruses in A549 - see graph.

iii) IP with anti-ADP serum didn't work (even in positive control). Next week going to try IP with controls and various sera to optimize experiment conditions.

EXHIBIT

C65



Plaque development assay, A549

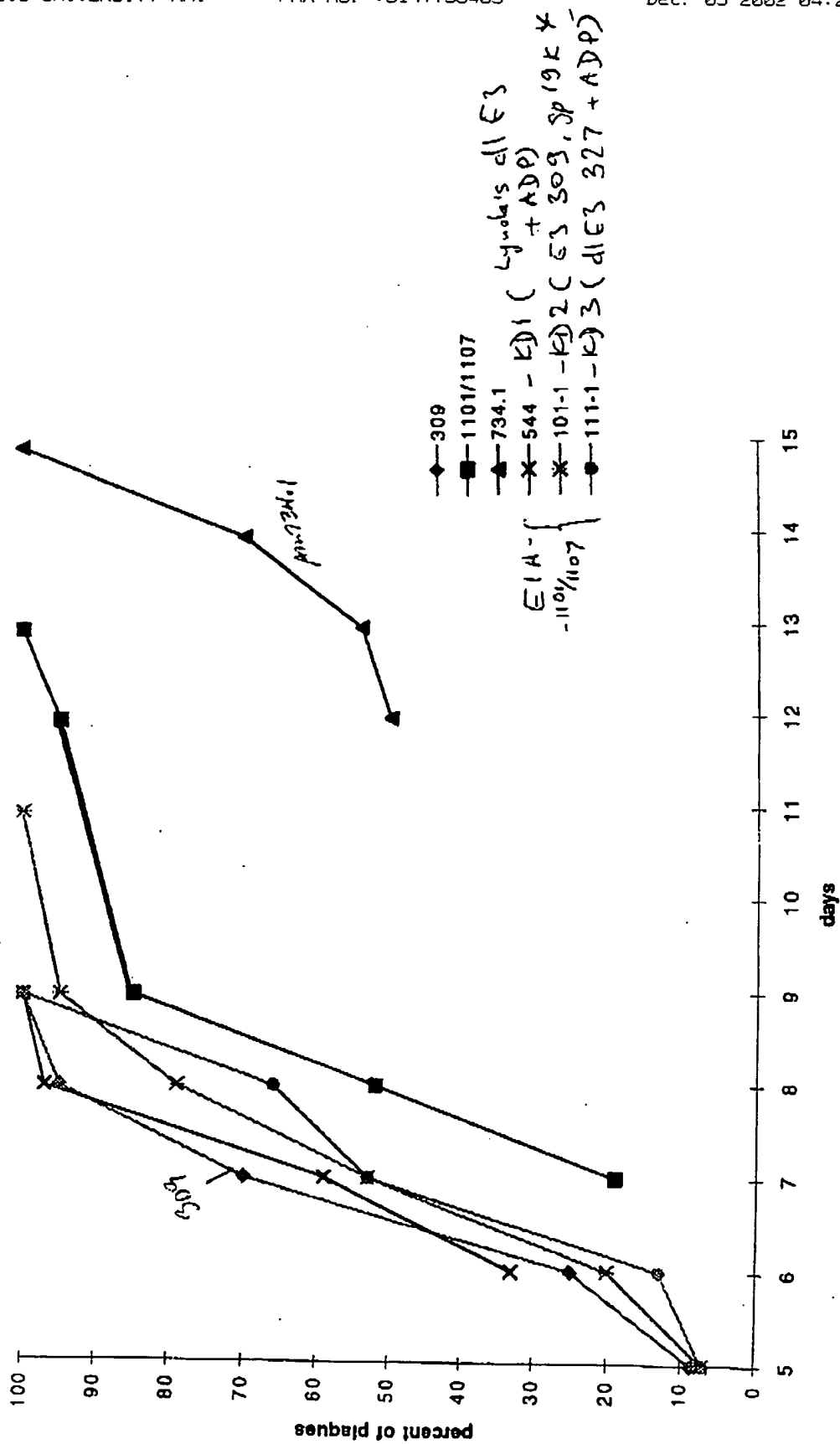
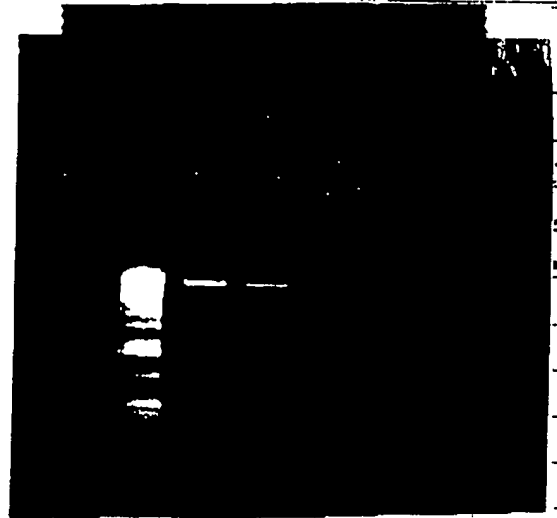


EXHIBIT  
C66

Mohan, 6/13/97

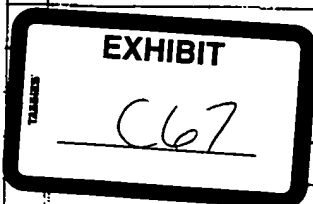
## 1. C.F. project:

- a) 293 cells expressing anti. by p2E1Sp1A/3.75pc Rep78 a viral DNA - shows the f slow.
- b) 293 < Rep78 cell lines sho. fragment by PCR.



## 2. E3 project:

a) Particles/OD, OPU



	OPU <sup>1</sup>	OPU <sup>2</sup>	Pfu
r700 =	$1.52 \times 10^{12}$	$9.8 \times 10^{11}$	$9.88 \times 10^{10}$
C+14.7 =	$1.9 \times 10^{12}$	$9 \times 10^{11}$	
GP19K =		$2.8 \times 10^{11}$	$1.2 \times 10^{10}$
ITR LacZ =		$2.2 \times 10^{12}$	$9.8 \times 10^{10}$

Pfu will be  $\approx 10^3$  reproducible

- b) Lact purified r700, C+14.7, and GP19K virus - 100  $\mu$ l  $\rightarrow$  viral DNA  $\rightarrow$  gel.

It seems from the gel, OPU/DNA ratio looks reasonable. ~~OK~~

If it is true then virus prepn - does it have more defective particles in C+14.7, or Toxic?

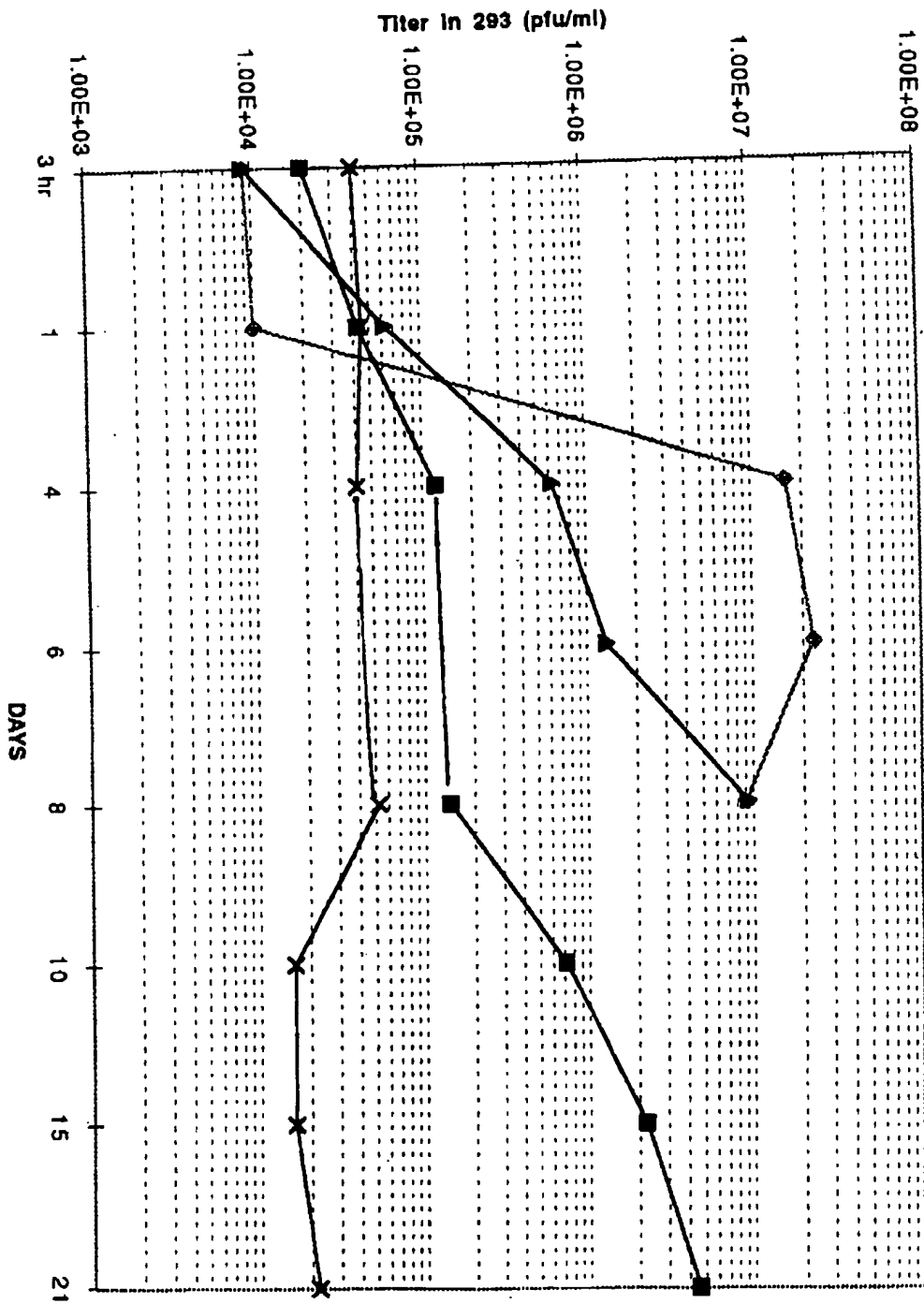
- c) I.F: A549 cells, r700, NP6-K 14.7 and GP19K (50 pfu/c) < 14.7.

- d) Transferring GP19K fragment from pCI19K to left hand plasmid p2E1Sp1A and p#30 (p2E1Sp1/VA1) #30 NP6K GP19K  $\rightarrow$  pCI. Have clone p2E1Sp1. I have a clone in p2E1Sp1A  $\rightarrow$  plasmid prepn  $\rightarrow$  I have clones in p#30 vectors  $\rightarrow$  analysing. VA1, VA2, LacZ opposite ends

3. Cancer project: HEL 299 cells, dl309 and dl1101/14.7 (50 pfu) See growth curve graph

Sheet1 Chart 6

Growth curve 500 pfu/cell



◆ d1309g  
 ■ d11101/07g  
 ▲ d1309ga  
 × d11101/07

EXHIBIT

C68

KANDY 6/12/5+

E3 proteins

- I did a Western with C+14.7 and VP6k147B
- I am doing an IF with Kostya's CMV E3 and Todor's 760 R10
- I have a problem with cloning, I am working on it.

FasL project

- I have ~ 15 new plaques, they came at about the same time as 12C2. I am growing them up.

Cancer therapy

- Time course in 293, A548 and MCF 7 is done, I am making 0 hour timepoints.

E2-Gal

- No plaques yet (not even in control).

EXHIBIT

C69

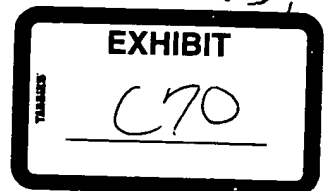


Kostya 6/17/97

# 1) E3 project.

← 734.1 ← E3

- i) Have made the left-hand plasmid based on p#30 (pΔE1Sp + leez-RSV) with CMV-E3 pm 734.1 expression cassette in it. Now making cell prep. to check for expression and making virus.
- ii) Failed to clone same CMV-E3 pm 734.1 into plasmid with Lynda's E3 deletion. Going to reclone CMV-E3 pm 734.1 into pCI 2xPac (Lynda), then I'll be able to put the expression cassette to PciI site of either Lynda's plasmid or pBK611.
- iii) Karol have shown that Terry's PC1/14.7 either don't express or express weak. I'm going to clone 14.7 from pMT2/14.7 to pCDNA3.1Zeo(+), then CMV-14.7 to p#30.



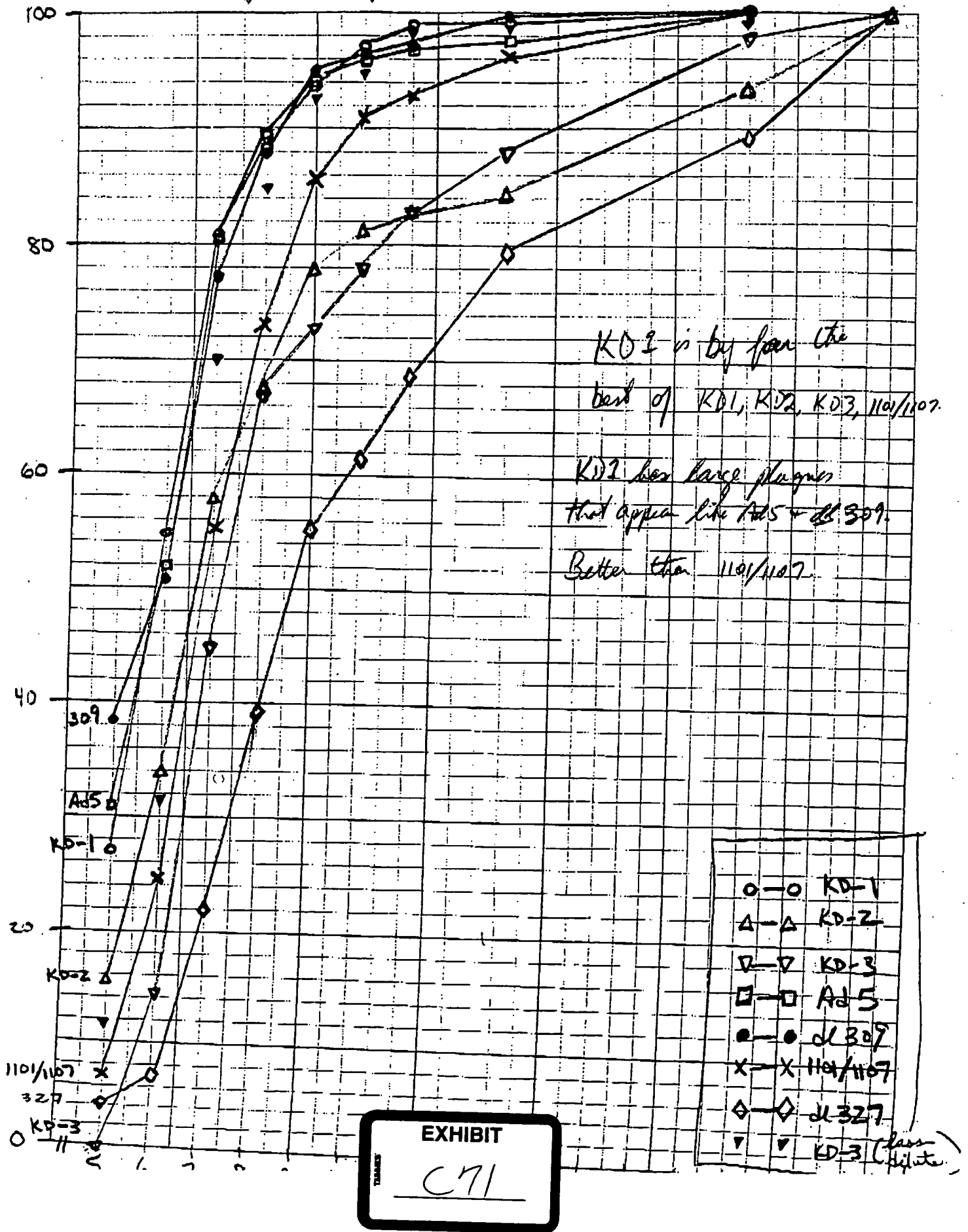
# 2) Cancer therapy project.

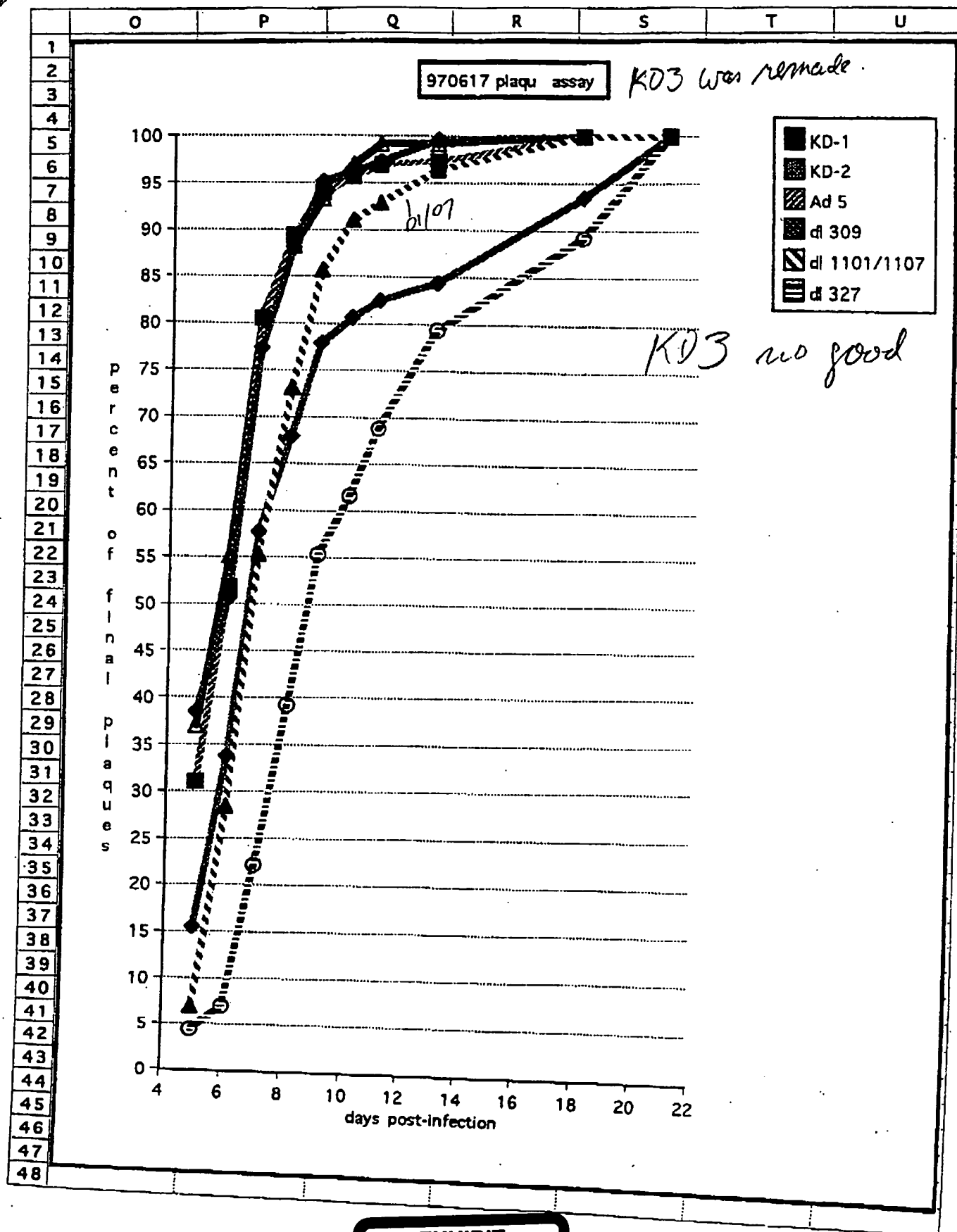
- i) Got 293/E4 from Valery Krougliak. Now growing them up to freeze and to repeat transfection experiments with SP-B ↔ E4 promoter substitution.

- Plaques are later than E4(+)
- Plaques are of different morphology (d1366 for me as well)
- Defect of linearization?

- ii) 293 cotransfection of p626 E4 ↔ CMV + d1366.
- 293 cotransfection with E4 expressing plasmids.
- iii) Ann's results with K13

## 970617 Plaque Assays on AS49





EXHIBIT

C72

	A	B	C	D	E	F	G
1	970617 PA						
2	KD-1	5	6	7	8	9	10
3		37	55.2	80.9	88.4	93.4	97.1
4	KD-2	5	6	7	8	9	10
5		15.6	33.9	57.8	67.9	78	80.7
6	Ad 5	5	6	7	8	9	10
7		31.2	51.9	80.6	89.5	94.1	95.8
8	d 309	5	6	7	8	9	10
9		38.5	50.8	77.5	88.1	95.1	96.3
10	d 1101/1107	5	6	7	8	9	10
11		7.1	28.6	55.4	73.2	85.7	91.1
12	d 327	5	6	7	8	9	10
13		4.5	7.1	22.3	39.3	55.4	61.6
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EXHIBIT

C73



	H	I	J	K	L	M	N
1							
2	11	13	18	21			
3	99.2	99.2	100	100			
4	11	13	18	21			
5	82.6	84.4	93.6	100			
6	11	13	18	21			
7	97	97.5	100	100			
8	11	13	18	21			
9	97.5	99.6	100	100			
10	11	13	18	21			
11	92.9	96.4	100	100			
12	11	13	18	21			
13	68.8	79.5	89.3	100			
14							
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EXHIBIT

C74

06/20/97

Kostya

1) E3 project

i) Started cloning of CMV-E3 pm 734.1 <sup>has zeo gene</sup> expression cassette to left-hand plasmid (p#30) and into E3 (in place of Lyda's E3 deletion).   
 → Clone into SPC expression cassette to TB animal.

ii) Started cloning of CMV-14.7 expression cassette from pE1-14.7 (Terry-Chip) to left-hand plasmid.

2) Cancer therapy project

Have no problems with SPB ↔ E4 promoter substitution. (293-TTPI Mohan)

I'm starting with Rep/E4, AS23/E4 from GT1, need to check them for ability to suppress growth of 366 (E4<sup>del</sup>).

W162 - (expressing E4) - if Ann can find them.

Growing up plasmids containing E4 under the control of CMV and native E4 promoters. (for cotransfection)

Valeri Kravchik wrote me that he would be able to send us 293/E4 (VK2-20) when Dr. Wold write him a letter with request. His address is on the next page.

TI-E4 deletion cassette.

EXHIBIT

C75

Karoly 6/20/97

E3 proteins

- I am growing up <sup>gpi19K, p28<sup>u</sup></sup>KT2 and <sup>gpi19K, p28<sup>u</sup></sup>KT9<sup>in E1</sup> into the 15 cm dish
- I am doing a Western with KT4 and KT6
- I am doing a time-course with KT7 and Ct (KT18?)
- I am cloning RID with CMV promoter into the shuttle vectors.   
 Have in Nippon's shuttle, Graham's shuttle.   
 RID from Ad2 and Ad5.

Fool project

- I am <sup>Have 2 plagues</sup> growing the ~~clones~~ <sup>plagues</sup> these plagues grow very slowly in 293/CrmA cells.   
 - MT2 promoter is in pCI2Pac (Lynda) (maybe; probably)

Crm A

I have Crm A in Nippon's shuttle plasmid, I am going to make virus with it.

Cancer Therapy

- I am fitting missing timepoints.

CMV-147 (Koty)  
CMV-gpi19 (Nohan)

We need maps and sequence of plasmids that they sent us.  
Koty will check.  
Especially the left hand plasmid with RSVTR/lacZ

Given to GTI 6/18/97  
pCI-RID(5) } Chip  
pCI-RID(2) }  
pCI-gpi19K

Will send to GTI next week.  
pCI-14.7K (expression)  
Send to GTI next week

EXHIBIT

C76



Valeri Krougliak,  
Assistant Professor  
Institute for Gene Therapy  
Mount Sinai Medical Center, box 1496,  
One Gustave L. Levy Place  
New York, NY, 10029  
Tel. 212-824-7748  
Fax: 212-849-2437  
E.mail krougv01@doc.mssm.edu

293 expressing EU (VK2-20)

Published in Human Gene Therapy 6: 1575-1586  
(December 1995)

Have 3 plasmids with ~~et~~ deletion.  
Cannot rescue virus.

EXHIBIT

C77

h/27/97 *Leslye*1) E3 project

i) Cloning of CMV-E3 pm 734.1 expression cassette into left-hand plasmid and into plasmid with E3 deletion is in progress.

ii) Terry's pCI-14.7. Made miniprep of the plasmid, it looks like this is pCI with Ad5 14.7 in it. Made maxiprep, Karl is checking it for expression. Now doing recloning of CMV-14.7 expression cassette from this plasmid to left-hand plasmid.

2) Cancer therapy project

Got A549/E4; Hep/E4; W162 from Ann. Doing plaque assay of dl366 on these cells with 293 and A549 as controls.

Going to try to optimize transfection efficiency for these cells.

Going to try transfection-infection experiment (infection with 366, transfection with CMV-E4, SPB-E4 & E4-Epromoter as a control) on 293 cells.

Going to try cotransfect 293 with 1101/1107/EOR1 + CMV-E4 plasmid).

293/E4 (Valeri Krongliak)?

EXHIBIT

C78

PINE 3.95 MESSAGE TEXT

Folder: MAIL Message 41 of 42 90%

Date: Mon, 23 Jun 1997 17:28:35 -0600 (CST)  
From: WOLDWS@SLU.EDU  
To: "KROUGV01@DOC.MSSM.EDU"@SLU.EDU  
Cc: WOLDWS@SLU.EDU  
Subject: Request for cells

Dear Valeri,

I hope all is going well with you. I'm happy to see that you have a faculty position in a good university.

I am writing to request the 293 cell line expressing E4 (VK2-20) that you published in Human Gene Therapy (6:1575-1586, 1995). We have several plasmids with deletions in E4, and we have been unsuccessful in rescuing them into virus. We would use your cell line for that purpose.

Thank you in advance.

Sincerely,

? Help	M Main Menu	P PrevMsg	- PrevPage	D Delete	R Reply
O OTHER CMDS	V ViewAtch	N NextMsg	Spc NextPage	U Undelete	F Forward

EXHIBIT

C79

970687

Gene Therapy Meeting

① Fast Ad

Karl

13 of Fast vector ~ 11 blue (23 plaques total)  
1 blue & titted MCP7 - CrrA

3 plaques by PCR do have Fast gene.

PCR - Fast in Nippon left and shoulder. pBHG 11

Next

5 from 60 mm dishes

Check by IF - all plaques.

Check by PCR for Fast.

②

Ad-14.7 PGK vector - expression at 2 d pi  
p734.1 - gp19 exp., 14.7k exp.

③

C<sup>+</sup> kills cells at 2 days pi.

C50 stock - strong discrepancy  
between particles &  
titers.

Raffinose → supernatant → perhaps some expression.

This plasmid expresses β-gal in transient/COS cells.

β-actin promoter & out enhancer; pol II for 14.7k.

This was done at 10<sup>8</sup> PFU (titer could be much higher)

Repeat using titer calculated from OD & Dmazarov's eq.

This was better than β-actin promoter enhancer in COS.

But tried to make vector from several diff promoters/polys;  
this was only one that gave plaques.

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

C80

TAMM

GEL 1997:06:27 12:46:35, Range - 0.01-249.86 Counts, 1.00x

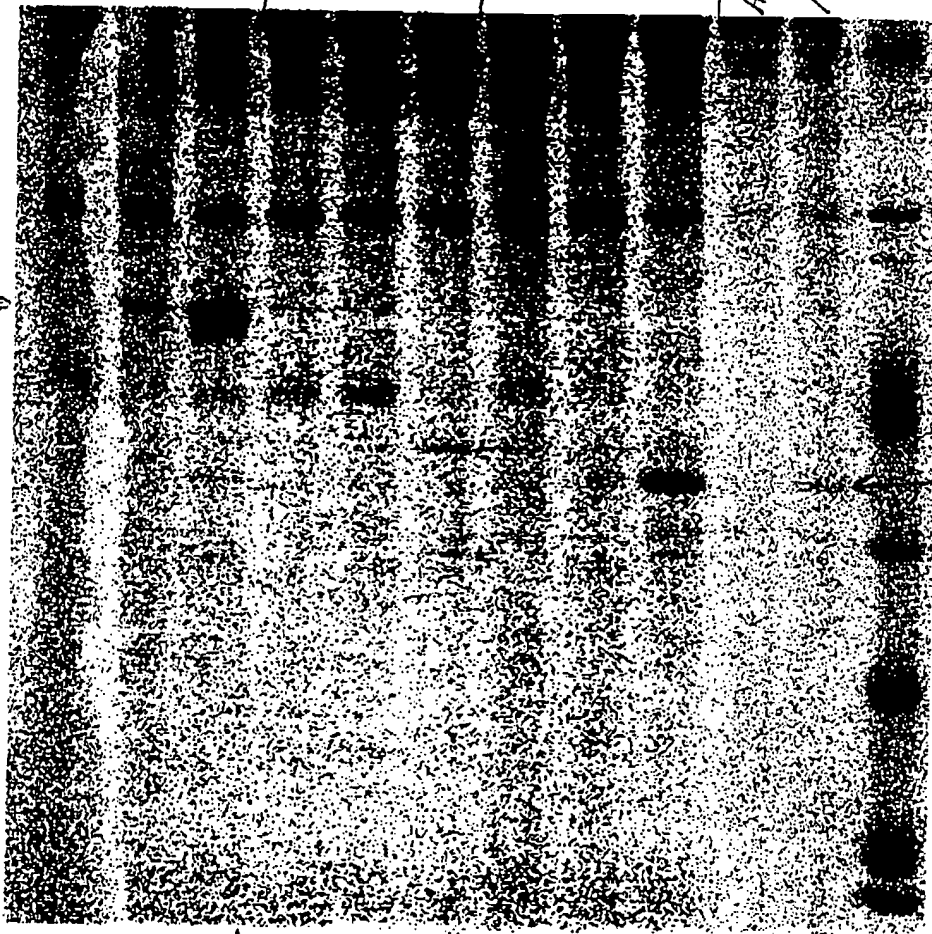
Karl

Transfection of p734.1 into 293

exposed overnight on PhosphorImager.

293	293 + 734.1	700 (200 pi)	293	734.1	700 (200 pi)	293	734.1	700 (200 pi)	AS219	AS219 + NPK-14.7B day 3 pi
-----	-------------	--------------	-----	-------	--------------	-----	-------	--------------	-------	----------------------------

9p19K →



14.7K in mud

α 9p19K	+	+	+					
α RIDB				+	+	+		
α 14.7							+	+

EXHIBIT

C81



Mohan, 6/27/97

1 C.F. project:

- a) 4 plaques (SPC Rep78 virus?) <sup>(very good CPE)</sup> are growing in 293 278 cells. 3 plaques showed CPE. 4th one is slow ~ wild type.
- b) RT-PCR for Rep78 antisense RNA were done <sup>This work</sup> employing RNA + Reverse Transcriptase to <sup>See graph</sup> verify the PCR fragment is due to RNA and not from DNA contamination. It seems 293 Rep78 cell line is expressing Rep78 antisense RNA.
- c) pSub VIII plasmid → chips → plasmid preparation. Elizabeth will make.

2 E3 project:

	Particles / OD = 0.8	
	pfu/ml	OD
x d1704	$2.4 \times 10^{11}$	$0.1 \times 10^{12}$
d1722	$1.35 \times 10^{11}$	$0.99 \times 10^{12}$
d1731	$1.44 \times 10^{11}$	$1 \times 10^{12}$
d1739	$7.7 \times 10^{10}$	$1.72 \times 10^{12}$
d1753	$1.07 \times 10^{11}$	$1.18 \times 10^{12}$
d1762	$1.58 \times 10^{11}$	$3.18 \times 10^{12}$

d1704 OPU is lesser than pfu. (Repeat)  
 - do  $10^8$  pfu +  $10^9$  pfu

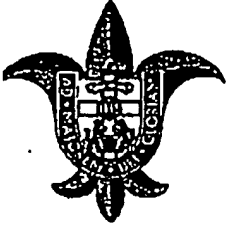
EXHIBIT

C82

- b) MCF-7 cells transfected by p2E1SpA/gp12K and p30 (Nimpen's) gp12K (#2, #3 & #7) got contaminated. (Yeast) Repeating again for I.F.

- c) Cloning Ad2, Ad5 RID into p2E1SpA and p30 is in progress (from Karl)

- d) RTI (14.7 LacZ) gave  $6.2 \times 10^6$  pfu → More CPE  
 Stocks → 5 tubes - 1 c.i.



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**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-19-02

From: William Weld

Phone: 314-577-8432

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Deliver to: Dan Kaster

Phone: Fax: 552-7305

Total number of pages including this page: 15

Message:

8 - 100MM

7/13

todo

phone:

Ab in whole / read T/c on Arms deck

Inventory virus bank \* 6/11 / 1/11

✓ (KBS from freezer) / when brought up

$2 \times 10^5 / \text{ml}$

Develop the gel (Monday)

✓ (cells frozen + thrown out) ✓

$7.5 \times 10^7$

10 less than 100%  
3mls / 250mls cell

Ad 1 + Ad 6 Virus stock (5mls of stock)

Set up  
1-100ml → stock

11.6 pre Absorbed. ✓

Set up \*

293 cells from Kang (Super 1) cell  
Gibco A549 III (earliest stock) (175 flasks)

VS103 5-2-1 stock 50ml → 100mm plate → finish

5-4-1 Spun down medium 20% FBS 100ml → 100mm plate

(Harvested) (960725)

Bayley

11/07/6 (911003)

104-8

30ml 2 medium plate

freeze / thaw

harvested (960725)

104-8

EXHIBIT

D1

9/4/96

CSC Banded the virus

Ad1

960825

1101/1107 Baileys (960802)

freeze / thaw

sonicate x 2

Vol after son 1101 < 21mls

Ad1 < 19mls

add  $V \times 0.51 = g$  of GCL

10.71g in 1101/1107 Baileys  
9.69g in Ad1.

infect 3 liter spinner with

dl 717 Vp 891208 Titer  $1.3 \times 10^{11}$

cell count 3 liter  $3.5 \times 10^5$

Vol = 3 liters (3000mls)

$3.5 \times 10^5 \times 3000\text{mls} = 10.5 \times 10^8$  total cells

use 20 pfu's / cell

162ul =  
use

$\frac{1.0105 \times 10^{11}}{1.3 \times 10^{11}}$

x 20 pfu's

KB's are healthy no clumping.

EXHIBIT

D2

9/17

~~9/17~~

1. Atlanta 6013E  
 $\frac{19}{4} \times 3 =$

first  
 (cells are shriveled up)  
 $1.4 \times 10^5$

\* 2. Brouhitatee  
 6m1228  
 $\frac{28}{4} \times 3 =$

cells look very health  
 $2.1 \times 10^5$

3. NYC Apa 4819  
 $\frac{31}{4} \times 3 =$

Nice + healthy cells very  
 full + round  
 $2.3 \times 10^5$

4. present 4m1938  
 $\frac{21}{4} \times 3$

Cells are dividing now  
 smaller cells but healthy  
 $1.5 \times 10^5$

5. NYC AF B4870  
 $\frac{22}{4} \times 3$

cells look great  
 $1.6 \times 10^5$

6. B10W 6m0741  
 $\frac{21}{4} \times 3$

Strange shaped cells  
 Rough edges not round  
 $1.5 \times 10^5$

infected 3 liter spinner with  
 1101 / 1107 20mls

$3.7 \times 10^5$  cells/ml

EXHIBIT

D3

September 18, 1996.

Cloning *EcoRI*-*XbaI* B fragment to pBSSK(+)/*EcoRI*+*XbaI*  
 of pFG140

Lanes : mixtures of

1) pBSSK(+)

2) - 9) clones

10) - 12) p21 (Mrippen's plasmid)

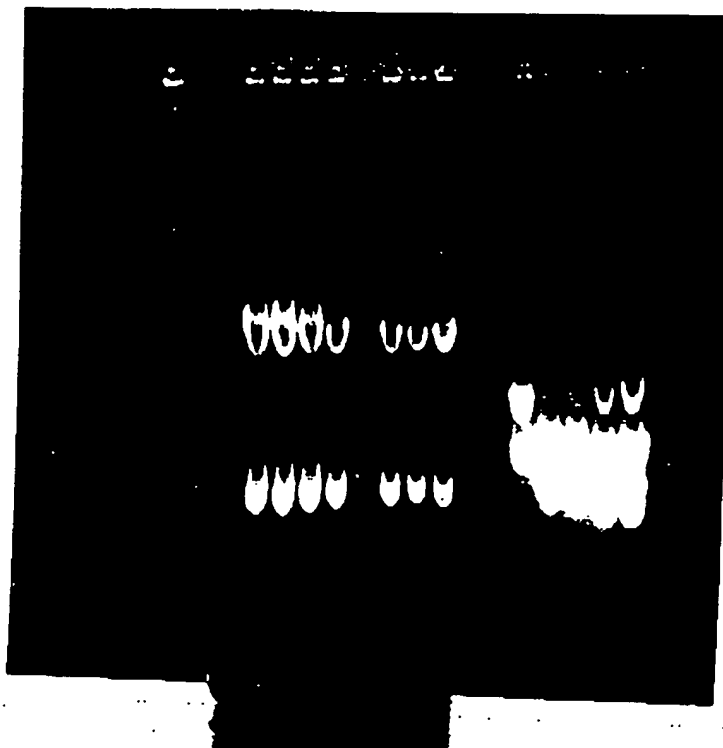
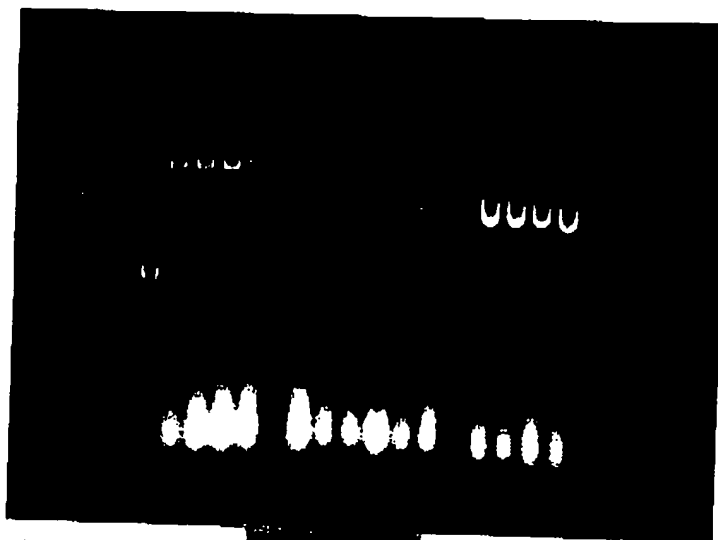
14) - 17) pISP-C3.7.

First experiment  
 09/18/96

Digestion patterns of  
 the plasmids.

- 1) Ladder
- 2) vector (pBSSK(+)) *R1/XbaI*
- 3) fragment B pFG140/*R1/XbaI*
- 4) - 11) - *R1/XbaI* clones 1-8
- 13), 14) - p21 (Mrippen) *EcoRI*
- 15) - 18) - pISP-C3.7 + *EcoRI*

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



EXHIBIT

D4

pBSKS(+) + 8 hr nt pK1/40 RT/16g

(pK1)

Lanes 1) Ladder

2) p1 + NdeI

3) Bst 1107 I

4) Sma I

5) Bst 1107 I + Sma I

nucleic  
core

⇒ size of R1 - Nde fragment?

⇒ RNA masked 600 bp pK1

Bst 1107 I - Sma I fragment

2% agarose gel

1) Ladder

2) pK1 / Sma I + Bst 1107 I

+ RNase

⇒ ~600 bp fragment

EXHIBIT

D5

Checking the distribution  
pattern of plasmas

1) Ladder

2) L1 / NHe + R1

3) L1 / Xe3

4) L2 / NHe + R1

5) L2 / R1

6) L3 / NHe + R1

7) L3 / R1

EXHIBIT

DL



28 September 1996.

minipreps

1) Ladder incl.  
2, 3, 4) - pLSP-B 2.2  
5, 6, 7) - L2  $\Rightarrow$   
 $\Rightarrow$  pLKHE2A + Bam  $\rightarrow$  end + Cla

8, 9, 10) - L3  $\Rightarrow$   
 $\Rightarrow$  PCR(LKH dP) Bam  $\rightarrow$  end E2A+

SPB experiment  
10/27/96

minipreps.

1) Ladder  
2) - 3) - L1  $\Rightarrow$   
 $\Rightarrow$  pTZAd - Xba.

22-141 50 SHEETS  
22-142 100 SHEETS

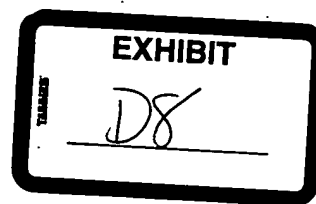
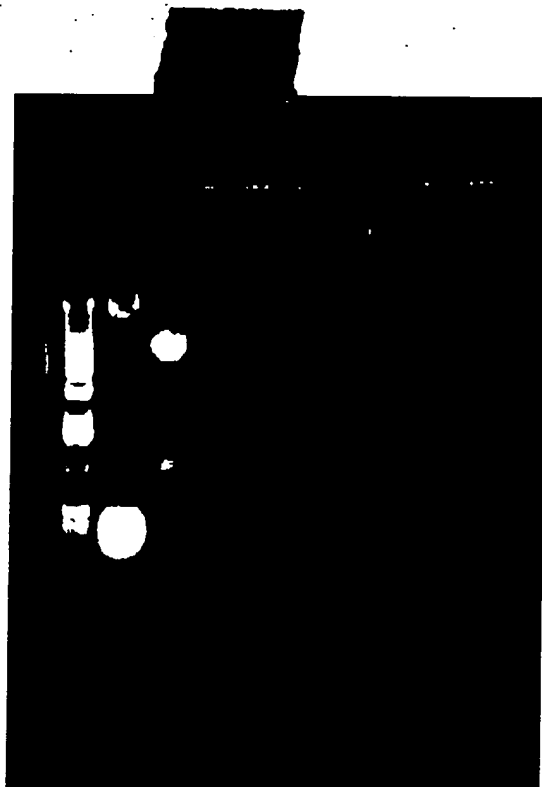
EXHIBIT

D7

September 30, 86.

Cloning of CMV promoter into  
Bst 1107I site of p21 (Nippr).

- 1) ladder
- 2) p21 / Bst 1107I
- 3) pCDNA3 / Bgl II + Bam HI



22-143 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

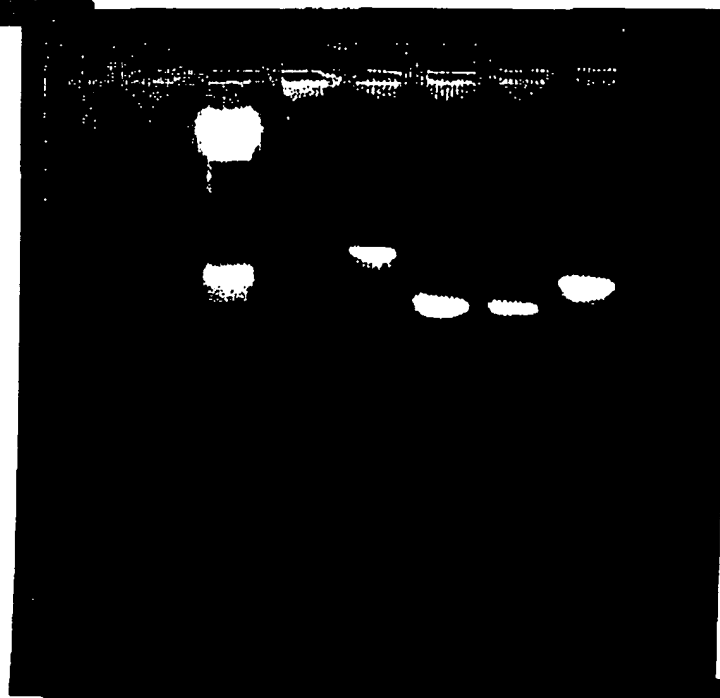


October 2, 86

Testing primers in PCR

- 1) Ladder
- 2) KJ2 - KJ3
- 3) KJ1 - KJ2
- 4) KJ4 - KJ5
- 5) KJ6 - KJ7
- 6) KJ8 - KJ9

→ all 9 primers  
are OK.



October 9, 96

Analysis of clones

1-2, 4-8 of

pK1 / Bst / NotI + SalI + CIP  
+ 19K\* (with mutation)

1) ladder

2) pK1 / RI + PacI

3) - 9) - clones / RI + PacI

clones 1, 4-8  $\Rightarrow$  recombinant $\Rightarrow$  clone 2 - wt. $\Rightarrow$  name recombinant  
plasmid (PK2)

October 21, 96

Analysis of miniprep  
restriction~~October 21, 96~~

1) pL2 / SacII

2) - 8) clones

1-7 / SacII

9) - ladder.

 $\Rightarrow$  1, 4, 7 - recombinant  
orientation?Cloning KDI ADP  
10/11/96

EXHIBIT

D9

October 27, 96.

Analysis of min prep  
by restriction1) ladder  
2) p21 / KpnI

3) - (4) clones

p21 / Bst + CMV prom  
Bst + Bam + Klenow →

⇒ clones 125 - 136 / KpnI

clones 25, 26, 33, 34  
the orientation that I need  
(allowing transcription)clones 27, 28, 29, 30, 31, 32  
35, 36 ⇒ other orient.+ clone 23 from  
after cloning is ok.15 - 26) clones p21 / Bst + PCR product -  
- promoter SP-B (500 bp) with Bst I sites  
on the ends

⇒ clones 225 - 236 / KpnI

⇒ clones 27, 28 ⇒ correct orientation

+ clone 23 from previous cloning

clones 25, 31, 32 ⇒ other orientation.

Cloned SPB prom.  
in E4 10/26/96

EXHIBIT

D10

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

$C_{pin} \approx 70 \text{ photos}/\mu\text{m}^2$

361V 1380 Sm.B  $\rightarrow$  to sequence 3ex (1912\*)  
 1741 kpt use left and (C) 3  
 200V 1981 Bst

Wednesday, October 30, 98.

- 1) 3ex clone 1, 4(?) - red preparation for sequencing
- ✓ 2) Purify ADP Xba fragment, purify pL1/Xba/CTP vector

Ligation: insert (ADP Xba) 0.15  $\mu\text{g}/\mu\text{l}$  vector L1/Xba/CTP 0.10  $\mu\text{g}/\mu\text{l}$

control 15  $\mu\text{g}/\mu\text{l}$  5  $\mu\text{l}$  vector

4 Experiment 15  $\mu\text{g}/\mu\text{l}$  insert 5  $\mu\text{l}$  vector

+ 5  $\mu\text{l}$  buffer (5X) + 1  $\mu\text{l}$  ligase  $\rightarrow$  1 hr room temp.

Thursday, October 31, 98.

- 1) 283 cells tomorrow will be ready to pass  $\Rightarrow$  prepare HEBS and  $\text{CaCl}_2$  What about 2x media?
- 2) Sequence pL1
- 3) Prepare red ADP Xba insert / Ligate, transform
- 4) Order primers for sequencing p21 inserts
- 5) first, repeat PCR with p21 pL1
- 6) Ask Nathan about TP - ring AdS

$$C = 7.75/\mu\text{l} = 80.78 \times 10^3/\mu\text{l}$$

Cloning KD3 ADP

EXHIBIT

D11

Sequencing

pk 2 (8p191c\*) → clone 1

- MIX — 8  $\mu$ l
- Template — 1  $\mu$ l
- Primer — 3  $\mu$ l  
(KD1 or KD3)
- $H_2O$  — 6  $\mu$ l

P.T. temp 96°C

96°C — 30"

50°C — 15"

60°C — (4 min!)

25 cycles. →

KD1, KD3

HEBS 2x

for 200 ml

- ▼ 2 g HEPES
- ▼ 3.2 g NaCl
- ▼ 0.148 g KCl
- ▼ 0.039 g  $Na_2HPO_4$  (anhydrous)
- 0.4 g glucose

final pH = 7.0.

 $CaCl_2$  2.5M

MW = 147.00

for 100 ml

36.75 g ▼


 22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

EXHIBIT

D12

Friday 1, November, 1986

- 1) Prepare HESBS,  $\text{CaCl}_2$ , test precipitate, filter
- 2) Run  $\frac{1}{2}$  of 3 pFU, <sup>cut with Xba</sup> <sup>run c. 1000, purify</sup> fragment, <sup>ligate</sup> <sup>transfect</sup> <sup>disk</sup>
- 3) Cut TP-DNA with  $\text{EcoRI}$ , check on gel
- no fragment? repeat, take HESBS for template!!!  
take other pFU polymerase!!!

Saturday 2, November, 1986

- 1) Set up cells on 6 cm dishes @ dishes
- 2) Prepare HEP (0.1 M), NaOH (0.1 N) for cell culture
- 3) Cut TP-DNA with  $\text{EcoRI}$ , run electrophoresis
- 4) PCR ADX6 with pFu, pFG440 as template
- 5) Filter sterilize  $\text{CaCl}_2$  carrier DNA (salmon sperm DNA).

PCR with pFu

100  $\mu\text{L}$  template (1  $\rightarrow$  1000  $\rightarrow$  10000) ●  
 2  $\mu\text{L}$  primer (4) ●  
 2  $\mu\text{L}$  primer (5) ●  
 180  $\mu\text{L}$  H<sub>2</sub>O ●  
 20  $\mu\text{L}$  bf. for pFu. ●  
 2  $\mu\text{L}$  pFu ●  
 4  $\mu\text{L}$  of dNTP. ●  
 200  $\mu\text{L}$

Sunday 3, November 1986.

gno PCR product, buffer pFu?

- 1) Cut pAg PCR product ADX6, with  $\text{XbaI}$ .
- 2) Transfection (pFG 100. — 20  $\mu\text{L}$  (100))  
     each on 2 dishes (TPDNA spl + carrier — 10  $\mu\text{L}$  (100))  
     (TPDNA spl + pL2 20  $\mu\text{L}$  (200 pL 2x60))

EXHIBIT

D13

Monday, 4, November 1996 (clavary small volume!)

- ✓ 1) Purify fragment ADP-obs, ligate, transfer OHSα.
- ✓ 2) When transferring OHSα, seed cells on SOB-agar for preparing competent cells.
- ✓ 3) Order primers for sequencing p21 insert.

Ligation  $\frac{\mu\text{g}}{\mu\text{L}}$  vect (L1) = 0.1/1  
 $\frac{\mu\text{g}}{\mu\text{L}}$  insert (ADP obs) = 0.01/5.

Ligation (promega)

control 1.5 μl vect + 8 μl H<sub>2</sub>O + 1 μl ligase  
 exp. 1.5 μl vect + 8 μl fragment + 1 μl ligase → 2h room temp

Sequencing failed → too low p<sub>ix</sub>!!!

Repeat with both primers 3x more template  
 3x more primers

mix → 8 μl  
 template → 1 μl  
 primers

13 = dilute 1/10  
 take ① - 2 μl (3)  
 ② - 4 μl (5)

+ 6 μl H<sub>2</sub>O (1)  
 + 4 μl H<sub>2</sub>O (3)

There are no E3 poly A sites in L2!

May be it can use fiber's gene pA?

Moreover, there are no acceptor splicing site in your construct, how can it be expressed?

Check electrophore on expression of inserts in Xle deletion of E3.

Splicing in Ad, esp. E3 splicing.



22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

EXHIBIT

D14



Tuesday 5, November 1996.

- 1) Repeat sequencing : 3ex 1 clone. primers 1 and 3
- 2) Feed clones new 4ex (NS clones) on sectors.
- 3) Prepare competent cells DH5 $\alpha$
- 4) Check ~~clon~~ master insert into p21 with other restriction sites, start clone it into p21.

Sequencing  $\rightarrow$  with primer 3 have a precipitate.  
with primer 1 precipitate is much less.

Wednesday 6, November 1996

- 1) Extract plasmids from clones 4ex (5 clones)  
check if they are right
- 2) 4) - from yesterday.

Thursday 7, November 1996.

- 1) Feed clones too lipids culture for miniprep with Wizard Plus miniprep kit for sequencing
- 2) PCR with pFU of ADP (primers 4, 5)

75  $\mu$ l - H<sub>2</sub>O  
1  $\mu$ l - template p21 (no.)  
10  $\mu$ l - pFU  
1  $\mu$ l - primer 1  
1  $\mu$ l - primer 2  
2  $\mu$ l - dNTP  
1  $\mu$ l - pFU  
10  $\mu$ l DMSO

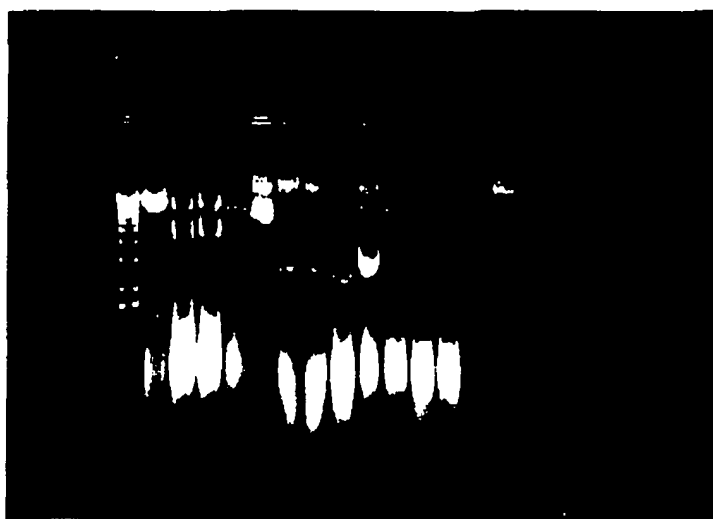
- 3) Prepare vectors L1/Xba and p2161 /  $\Delta$ gol1 -  
 $\rightarrow$  large fragment
- 4) Check env in p21 (Nde I)
- 5) Check new inserted DNA for MW?
- 6) PCR of inserts into p21, with SP-B it is possible to check orientation!

EXHIBIT

D15

hru 11, agree xel electrophoresis.

- 1) - colder
- 2) - 21
- 3) - 123 Juel (Butter D, orange)
- 4) - 125
- 5) - L1
- 6) - 10) 1-5 clones exp.
- 11) - 14) L1 / R1 prep
- 15) - pB4611 / R1 prep.



Conclusions!

1) it's on with chv  
monday 1/10/02

2) it's on with  
pB4611 R1

{ 1) L1 clones → trash  
2) L1/10 → trash

November 10, 1996, Sunday, November 11, 1996, Monday

- ✓ 1) Split 293 cells on new flasks.
- ✓ 2) Overlay 293 dishes with neutral red.
- ✓ 3) Run electrophoresis of Wizard minipreps and L1/R1.
- 4) PCR of clones + electrophoresis.
- 5) CIP pB4611 / R1, cut from the gel?
- ✓ 6) Put p21/GMV with EcoRI, cut the fragment from the gel?
- ✓ 7) Change the medium in 293 flask.
- 8) (New prep. of L1) Clean up old preps?
- 9) Send 54, 57 and L1 for Wizard miniprep!



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

D16

Tuesday 12, 1986

Cannot cut 3pm after Wizard kit + miniprep!

- 1) 123 - with EcoRI - pent, take from gel  
2) L1 - with XbaI - large fragment!

Wednesday 13, 1986

- 1) CIP miniprep, cut fragment from gel, cut fragment 123/R1  
2) Try to cut L1 with XbaI, if not successful → seed L1  
3) Ligate from 1)

12 pl vec + 5 pl vec + 1 pl vec  
+ 5 pl vec - control

L1/XbaI not in single band → 100% digest with XbaI  
→ extract with Wizard → seed it in ligase culture

pBAG 11/R1/CIP - A - fragment - not single band →  
be ready to distinguish between all this  
forms.

Thursday  
Friday 14, 1986

- 1) Transform DH5α with ligase mix from yesterday  
(Try to obtain high efficiency!!!)  
2) Extract L1 with Wizard, cut XbaI, run together  
with yesterday's (1) XbaI (one) on the gel.  
→ Try PCR! Maybe you can use it for screening?!?!  
→ Sequencing with new batches of 31!!!

PCR (Taq polymerase)

for 150 pl

- 15 pl buffer  
3 pl dNTP  
4.5 pl MgCl<sub>2</sub>  
1.5 pl Taq  
130 pl H<sub>2</sub>O

- 1) 123  
2) 125 (primers 10, 11)  
3) 223 (insert in p21)  
4) 227 (primers 8, 10)  
5) p21 - control (-) (primers 9, 11)  
6) p21 - control (-) (primers 8, 10)

19 54 (primers 5, 7)

- 1) 52  
2) pL2 - control (-)

12 reactions

15 (10-11)  
15 (10-11)  
15 (10-11)

(13, 14, 15) cut 54, 57 with PstI  
L? - control

225 with BstI 10/11  
427 p21 control

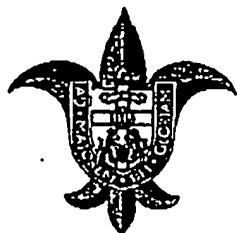
EXHIBIT

D17

FROM : SAINT LOUIS UNIVERSITY MMI

FAX NO. : 3147733403

Dec. 19 2002 11:23AM P1



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**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-19-02

From: William Wald

Phone: 314-577-8432

Fax: 314-773-3403

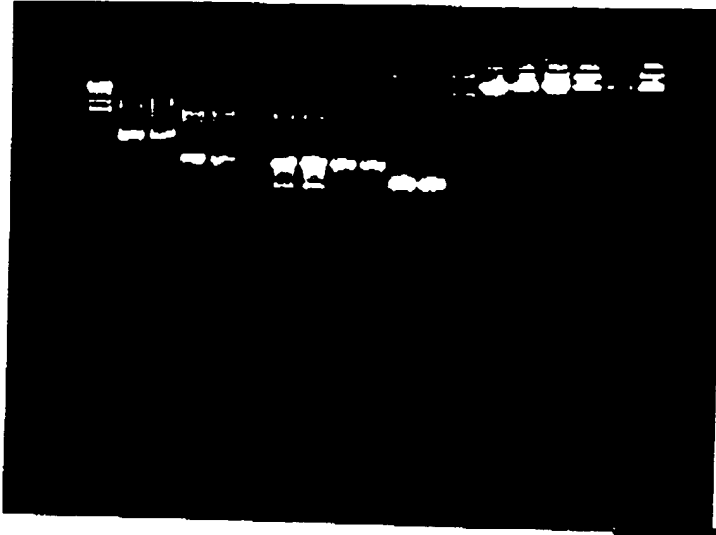
Deliver to: Dan Kasten

Phone: \_\_\_\_\_ Fax: 552-7305

Total number of pages including this page: 15

Message:

See explanation  
of the picture  
on  
previous  
page.



Conclusions: everything is fine  
except that Anti/62I didn't cut DNA  
(because of buffer 4, I think).

Friday 15, 1996.

- 1) ~~Transform L1~~! Clean up L1 preps., try to cut with Xba
- 2) Seed clones of pRK611 + 123 (cur).

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

D18

HAL 293 WI 38

Friday (1:00 /

Monday, 18, 1996

✓ 1) Analyse clones (1-24) pBUC11/A1 + CMV (128 clones)  
Run on gel and then cut with NdeI

✓ 2) Phage about SrfI

✓ 3) Transform L1

✓ 4) Sequence PCR

✓ 5) Prepare TP-DNA, TP-DNA/RI, Ad5 DNA, Ad5 DNA/RI

✓ 6) Split 293 to dishes for transfection

Sequencing

✓ 8 µl pre mix

✓ 5 µl p31 (0.5x)

✓ 1 µl primer (V) 1)

✓ 6 µl H<sub>2</sub>O

✓ 20 µl oil

circling

✗ pBUC11 gave 2 small fragments instead of 1.  
~~what~~ what is it?

clones gave incomplete digestion → O/N NdeI  
after PEG precipitation

Thursday, November 19, 1996

1), 3), 5), 6) from yesterday

if fail in 1), do PCR with 10, 11 primers!

Freezer at CR room 3 shelf from top

left drawer

upper box (-4)

tube with 918 in cap

(VS20) on side

→ Ad5  
CSA

EXHIBIT

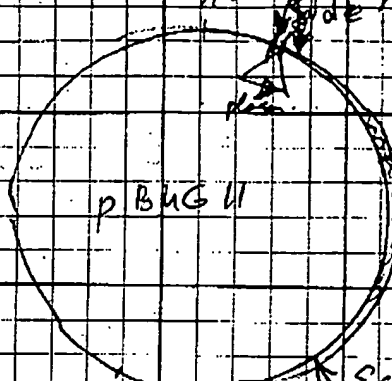
D19

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



RS XS here if placed based on pR

0/100 side? (in place of de EU)  
reasoned part ~ 2664



Nde I fragments

~ 18000  
~ 8540  
~ 2360 7540

Nde 31089  
DEE3 (3000)

R1 A fragment of (R3 (p21 + cm))

1 ch. ship  
cmv.

correct or

wrong orient

Nde (?!)  
Nde

Nde 31089  
R1  
Nde (?!)

Nde 31089  
Nde 27331  
Nde 13549

cmv - pae  
Nde 31089  
Esore 27331

Nde 13549

~ 18000 same as wt  
~ 7669 same as wt  
~ 5000  
~ 3565 instead of 4665

8282  
~ 18000 same as wt  
~ 7669 same as wt  
~ 5000 same as wt  
~ 3565 instead of 4665

1- Radder  
2- cmv  
2.6- cmv  
50 13

~ 18254 same as wt  
~ 8540 same as wt  
~ 5000  
~ 3540

~ 18254 same as wt  
~ 8540 same as wt  
~ 5000

34589

EXHIBIT

D20

← But it is not like this with ~~EcoRI~~ and ~~NdeI~~  
 because you were able to see  
 & It's like this, leave NdeI digestion of p21  
 gave linear form.

⇒ Incomplete digestion? (with NdeI)

⇒ cut with EcoRI  
 and PacI + BamHI (pBHG 11. as control).

1) ladder 2) pBHG11 / NdeI  
 minipreps: (6 ex)

3-26) - clones 1-24

⇒ clones 4, 7, 9, 14, 18, 21  
 → trash.

clones 1, 2 and other  
 with same pattern are  
 with correct orientation of  
 insert?!

Partial digestion with  
 NdeI

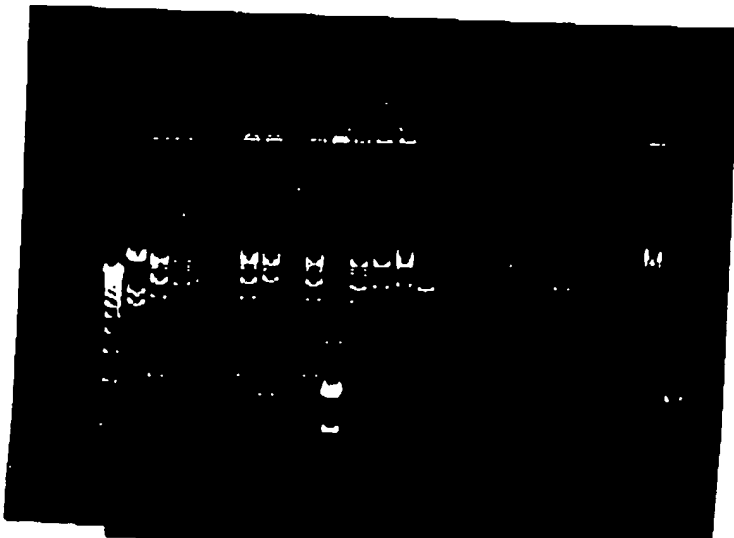
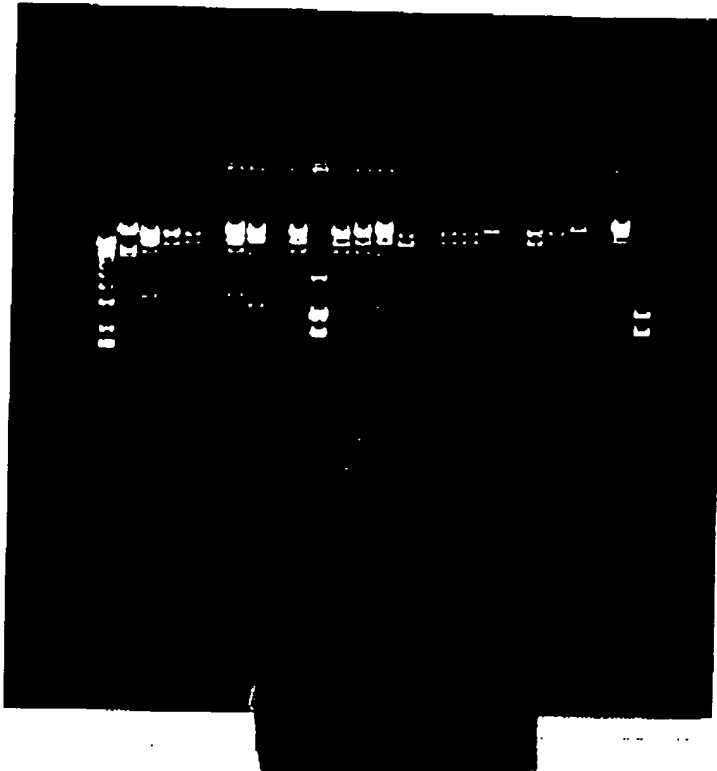
Bottom picture is the  
 same.

Or cloning in partially  
 digested pBHG11?  
 or star activity digested?

take to analyze RI

1, 3, 5, 6, 17, 19, 21

50 SHEETS  
 22-141 100 SHEETS  
 22-142 200 SHEETS  
 22-144



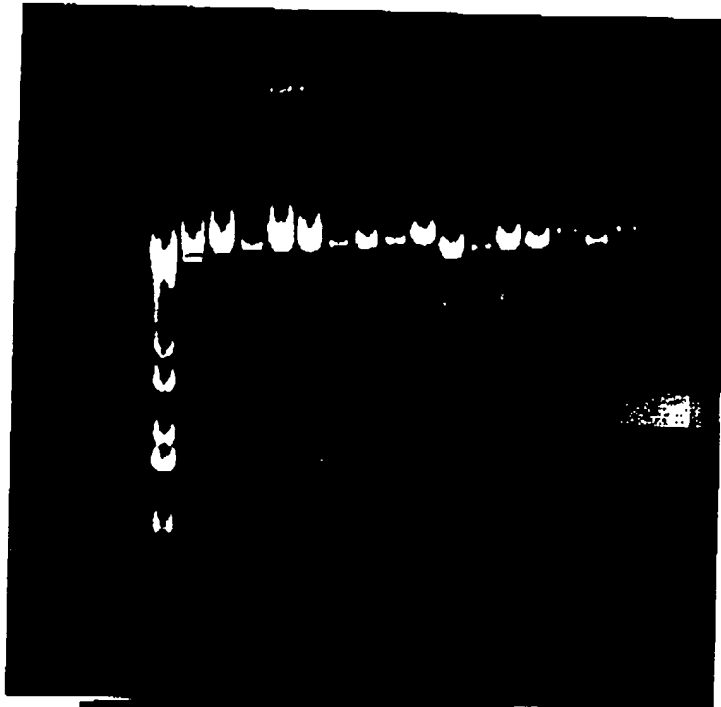
EXHIBIT

D21



Pac + Bam → NEB Buffer (4) - green. both enzymes

~~NEB Buffer (4) - green~~  
Eco RI - (Multi core) - Buffer 4, Promega



1) Calder

2) BUG / R1

3) 1

4) 3

5) 5

6) 4

7) 12

8) 18

9) 21

(10) BUG / Bam + Pac

(11) 1

(12) 3

(13) 5

(14) 6

(15) 12

(16) 14

(17) 21

(18)

Conclusions! 17, 21 are OK by Eco RI digestion  
others are result of Eco RI star activity  
when preparing vector.

Pac + Bam → pBUG II gave more fragments than  
expected.  
all clones are similar and different  
from pBUG II. Wrong orientation?  
They have different sizes than  
Pac 12 not work in clones?

17, 21 are not OK inserts are too small 11/27/02

EXHIBIT

D22

2 orientations

10000

11000

8000

7500

5000

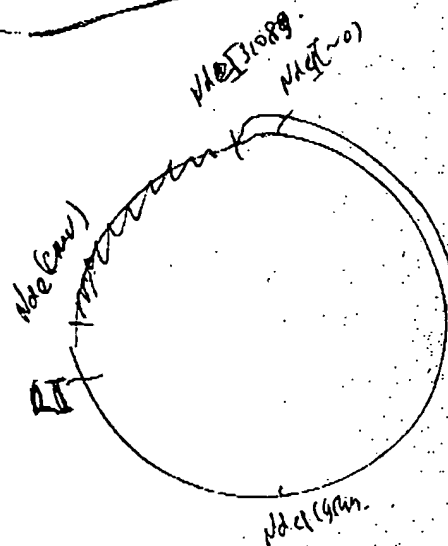
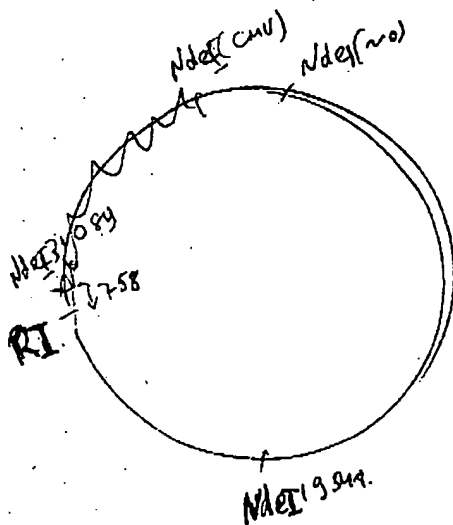
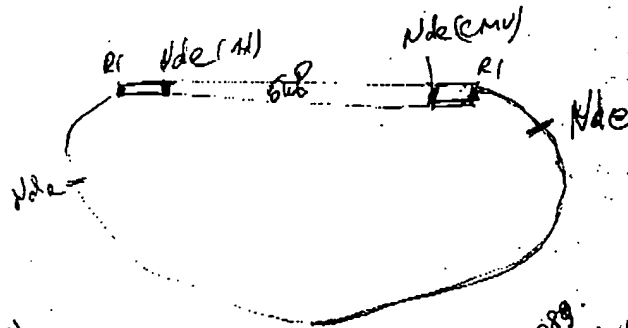
5000

3000

2700

you can explain  
it just in the case  
of larger fragments  
are result of  
partial hydrolysis

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

D23

if it is mixture of 2 plasmids  
and  $\Rightarrow$  there are just 2 Hae sites?

EXHIBIT

D24

Wednesday, November 20, 1996.

Thursday 21 November

- 1) Seed L1 for mini (mini) prep. with Wizard!
- 2) Cut TP-DNA Ad5 with  $EcoRI$ , check on G/E, dialize against TE.
- 3) Precipitate Ad5 DNA, check amount, cut  $EcoRI$ , phenol, precipitate.
- 4) Split 293 to dishes for transfection.
- 5) Sequence clones.
- 6) Prepare new vector pBHGHI/RI/ZIP, ligate with 12S/RI insert.
- 7) Seed 17,21 clones for further characterization.
- 8) Cut pBHGHI with RI, Nde, Bam, Pst, Bam + Pst? (with Pst)
- 9) Check  $SbfI$  site in pEG140, pBHGHI, p1, something?

Friday 22, November 22, 1996.

- 1) Transfection of 293 with pL2 + TP-DNA Ad5/RI or ~~AD5/RI~~
- 2) Extract 17,21 and L1 with Wizard miniprep.
- 3) Sequence clones.

seq #2 3 ex, clone 1 (31) - primer 10, 2.

seq #3,4 2 ex, clone 23 (223) - primers 10, 11

seq #5,6 1 ex, clone 23 (123) - primers 10, 11

seq #7,8 5 ex, clone 4 (54) - primers 6, 7.

8  $\mu$ l premix  
 5  $\mu$ l template (0.58)  
 1  $\mu$ l primer  
 6  $\mu$ l  $H_2O$

40  $\mu$ l oil  
 circling

EXHIBIT

D25

Monday November 25, 1996.

- 1) Cut new preparation of L1 with Xba, check on C/F
- 2) CIP pBAC11/R1
- 3) Check amount of AD5/R1
- 4) Cut 17,211 with Bam + Srf, pBAC11 - control → nt fragment 5858
- 5) Check plasmids with Srf I.

Spe I - site → 250 in CMV promoter.

⇒ in right orientation → fragment ~ 5800 bp

⇒ in wrong orientation fragment ~ 1000 bp.  
wt - linear form.Bam  
Srf  
clonase

- L1/Xba

- pBAC11/R1  
- AD5 (203)

1) ladder

2) pBAC11

3) clonase

4) clonase

5) L1

6) L1/Xba

7) L1

8) pBAC11/R1 (buffer H)

9) AD5/R1 (203?)

Promega  
buffers

EXHIBIT

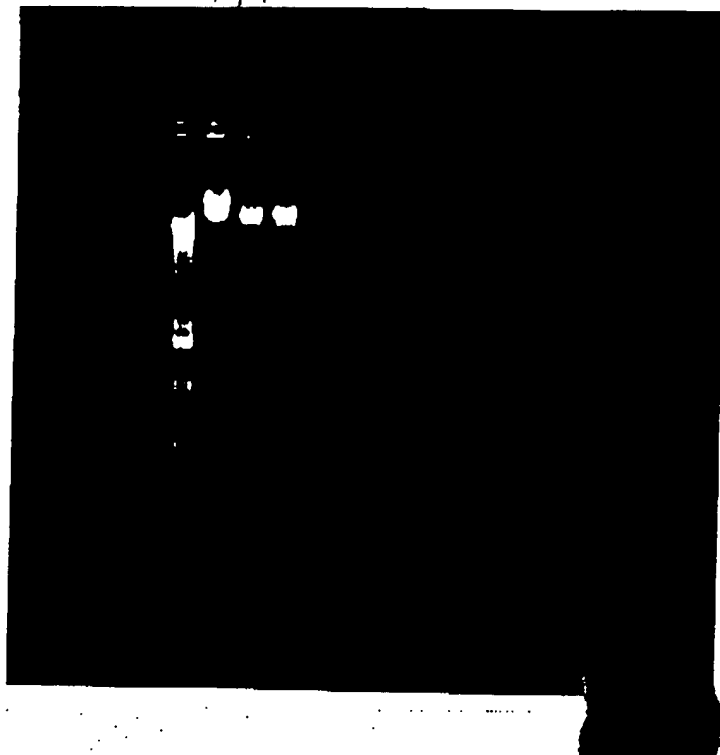
D26

Bam + Srf hydrolysis (buffer H) - strange  
mixture, same as before Bam + Pae → M.B.  
2 Bam sites or star activity?

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 046119  
- done 17  
- done 21



1) ladder

2) 04611A

3) done 17 Spe I

4) done 21

there are no fragments  
~ 6000 bp →  
→ wrong orientation  
or what?  
→ repeat cloning.

EXHIBIT

D27

Thursday, November 27, 1996

1) pBAC11/CIP → extract from gel  
 L1/Xba → CIP → extract from gel  
 ligate to inserts o/n 3(PCR)/Xba → extract from gel (2%)

2) PBAC 11/CIP (new prep, 0.1/1) → 10 µl  
 1,2,3 insert (old prep 0.05/5) → 7 µl

2 µl buffer  
 1 µl enzyme

o/n 16°C

11) PL1/Xba/CIP (new prep, 0.1/5) → 12 µl  
 3(PCR)/Xba insert (new prep 0.1/5) → 5 µl

→ 2 µl buffer  
 → 1 µl enzyme

o/n 16°C

Wednesday, 28, 1996

1) Transform DH5α cells with ligation mixtures from yesterday, try to obtain high efficiency!  
 plate cells in duplicates, 4/10, and all

2) Plan scheme of experiments to obtain Berger plasmids, check everything, start cloning.

3) Split 293 in flask

Friday 30, 1996

1) Seed clones 6ex, 9ex in liquid culture and agar plates

2) Split 293 cells, overlay 293 dishes with transfection

EXHIBIT

D28

Saturday, November 30, 1996.

1) Extract hex 25-36 with Wizard kit, cont with  
SpeI Extract w/ 1-24 with ligation,  
cont with Sac II.

2) from yesterday. Sec II - buffer C / orange  
SpeI - NEB Buffer II

3) Check references about splicing in Ad, especially  
in ES and fiber!

4) Start with genes IL and TC?

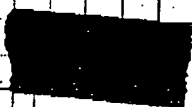
\* Filter REBS and cell 2 from freezer 2 time?!

WB

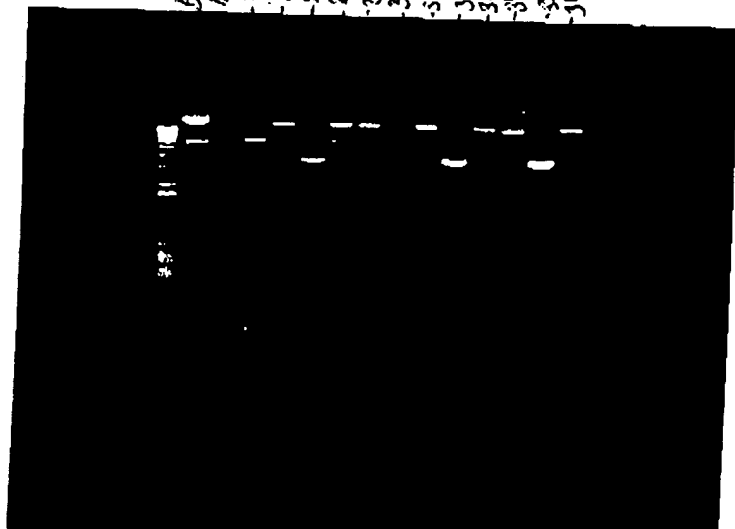
There is no SpeI site in CMV promoter according  
to my sequence  $\rightarrow$  may be clones 17 and 21  
from previous cloning are OK?

Monday, December 2, 1996.

- 1) Check new hex clones with EcoRI, select recombinants
- 2) Check new hex clones with XbaI, select recombinants



Bug 11/11  
25/11/11  
21  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36



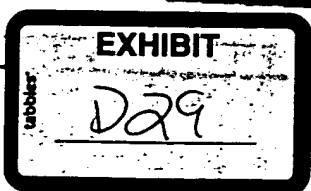
1)

- 1) Ladder
- 2) Bug 11/11
- 3) 25/11/11  $\rightarrow$  A fr.
- 4)  $\rightarrow$  25-36

Recombinants.

26, 28, 29, 31, 33, 36.

c) Can't see  
fragment  
on XbaI hydrolysis.





3)  $\text{Gex}$   $\text{SacII}$  (1 site in ADP), no sites in C1  
 + RI (0 sites in ADP), 1 site in C1  
 expected fragments correct orientation ~1261  
 wrong orientation ~1561  
 orange buffer (C)

4)  $\text{Gex}$  clones  $\text{Pac}$  +  $\text{Bam}$ .

1 h  $\text{Pac}$  (buffer NEB1 + BSA)

↓ heat inactivate

↓ + 1x buffer (E) +  $\text{Bam}$  → 1 h → E/K  
 (E)  
 (D)

NEB 1 - 10 mM Tris propylene-HCl, 10 mM  $\text{MgCl}_2$ , 1 mM DTT (pH 7.2)

$\text{BamHI}$  - 150 mM NaCl, 10 mM Tris HCl, 10 mM  $\text{MgCl}_2$ , 1 mM DTT (pH 7.9)  
 unique NEB buffer.

buffer D - pH 7.9, Tris HCl - 6 mM,  $\text{MgCl}_2$  - 6 mM, NaCl - 150 mM  
 orange D - yellow, pH 7.9

Buffer C (orange) pH 7.4, 10 mM Tris HCl, 10 mM  $\text{MgCl}_2$ , 50 mM NaCl

McCore (white) pH 7.8, Tris HCl - 25 mM  
 acetate

100 mM K acetate, 10 mM acetate

Take orange 2X?

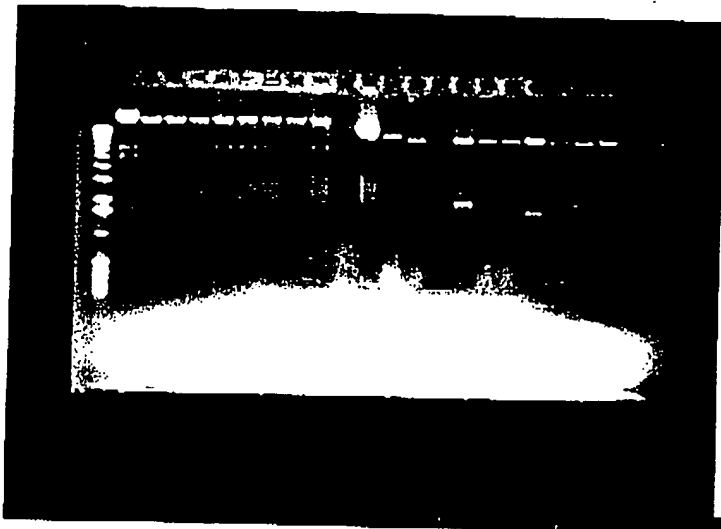
22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

EXHIBIT

D30

Cloned  $\lambda$  D3 AOP  
12/2/96

- 1) ladder
  - 2) pM11 / Pac + Bam
  - 3) 17
  - 4) 21
  - 5) 26
  - 6) 28
  - 7) 29
  - 8) 31
  - 9) 33
  - 10) 36
  - 11) - blank
  - 12) L1 / Sac II + R1
  - 13) - 241 - clones
- hex (1-12) / Sac II + R



the same gel,  
higher magnitude.

Conclusions:

- hex -
- clones
  - 2, 5, 6, 7, 8, 10 -
  - correct orientation
  - clones 4, 9, 12 -
  - wrong orientation

hex - absolutely  
unclear  
what is happening.

yellow ←

Tomorrow try to cut with Nde I,  
take 10  $\mu$ l of plasmid, good activity  
of Nde I.

Try some other enzymes? (Not Pac,  
Not Bam)

universal  
IX.

for example Spe I, Srf I.  
correct or ⇒ 448

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

D31

tabbles



1402 South Grand Blvd.

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**SAINT LOUIS  
UNIVERSITY**

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12-19-02

From: William Weld

Phone: 314-577-8432

Fax: 314-773-3403

Deliver to: Dan Kasten

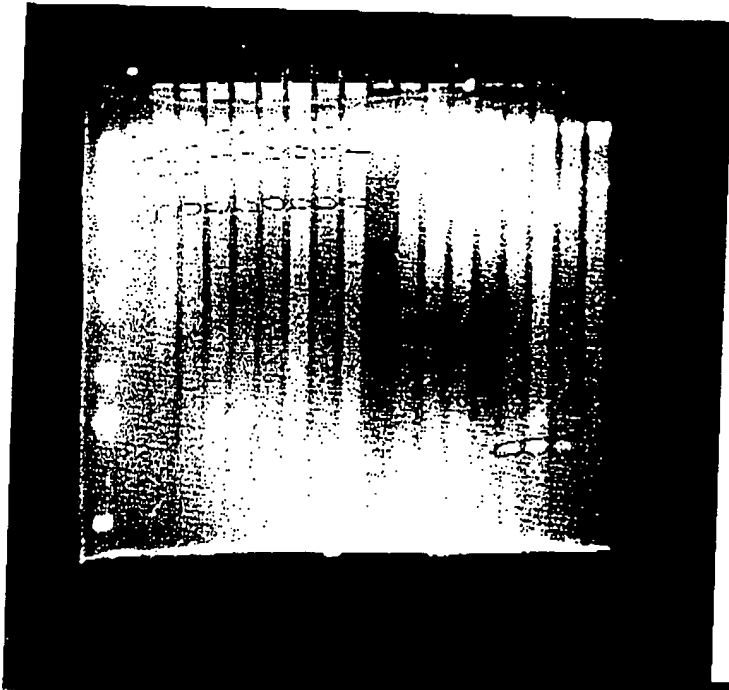
Phone: \_\_\_\_\_ Fax: 552-7305

Total number of pages including this page: 18

Message:

Tuesday, November 3, 1996

1) cut 600 clones with Nde (Bsp. I), yellow  
and pRF + Spe I (Bsp. universal 9X)



Conclusions 17, 21, 29, 37, 33 - one orientation  
26, 28, 36 - other orientation

If consider the previous picture, 26, 28, 36 → correct orientation  
because there was additional 6 bp from Bam  
fragment

Obviously there is Spe I site in CMV  
didn't give fragment because they are 17, 21  
wrong orientation (on Spe I hydrolysis)



Wednesday, 9, 1996

1) Cut Gex clones with SpeI. (buffer Multicore)

1) ladder

2) pB4611 / ~~SpeI~~ SpeI

3 - 100

17, 21, 26, 28, 29, 31, 33, 36  
- SpeI

there is fragment

6 kb in (26, 28, 36)

⇒ correct orientation

there is fragment

1200 bp in 29, 31, 33

⇒ wrong orientation

17, 21 - junk! Look  
also to upper fragment  
on the previous picture.

Conclusion: When everything is OK, it's OK.

Friday, December 8, 1996

1) Cut pFG140 → BamHI + NdeI fragment (9497)  
(contains SacI site, other  
fragments do not)  
other fragments  
- ~ 2500 - if there is no  
NdeI in pMX2  
- 2043  
- 1200  
- 6000  
if there  
is NdeI  
site in pMX2.

at 293 (pT1508) with NdeI + BamHI - vector

2) pT1508 293 cells

3) ~~Reseed~~ Reseed clones to new dish

4) ~~Grow~~ Grow

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

D33

Monday, December 3, 1996

1) and 3) 4) from Friday

2) Prepare LB for preparation of ~~growing~~ of ~~6ex~~  
Inoculate LB overnight (15h)

3) Prepare the HRP pH (8.5) (8.0) (7.5) (7.2),  
autoclave

4) can use 1101/1107 for ~~contamination~~ to attempt  
to obtain a virus with E4 promoter  $\rightarrow$  SP-B promoter  
substitution.

4) Start cloning EcoRI-SalI fragment pFG140  
into pK2 (gp19C+)

pFG140  $\rightarrow$  BamHI-Nde - Buffer)  
 $\rightarrow$  Sal - EcoRI - Buffer)

223 (p21 + SP-B promoter) - Nde - BamHI - Buffer) - vector  
pK2 (31, gp19C+) - EcoRI - SalI - Buffer) - vector

pFG140 Sal - EcoRI - expected fragments:

17869  
10585  
6905  
379

There is no Sal in pK2, if there is Sal, then instead of 17869 I will have ~9000  
that is what I need contains BamHI site!

There is Sal, then instead of 17869 I will have ~9000  
~8869

Wednesday, December 12, 1996

1) Extract max prep of 6ex (clone 26)

2) Extract Wband 4ex (clones 2, 5)

3) Prepare vectors and fragments from yesterday  
+ 4ex (2) Sal + RI

4) Check n2, 45 with XbaI; RI + SacII (sequence 11)

EXHIBIT

D34

223/ Bam + Wde  $\rightarrow$  1 m-2 Bam soon only ??  
 Bam HI star activity? M.S. there is 2 site  
 for Bam HI?  
 It looks like  
 there is 2 Bam HI  
 site  
 It also can explain  
 fragment on Bam a something  
 P.B.G. (1) and deplete by  
 liso

223/ (10 KB) (0.1 / 10)  
 223/ (10 KB) (0.1 / 10)

10  $\mu$ l vect + 10  $\mu$ l insert  
 10  $\mu$ l vect + 10  $\mu$ l H<sub>2</sub>O + 2  $\mu$ l AT + 1  $\mu$ l DMSO

**EXHIBIT**

1035

Friday, December 12+1, 1996.

- 1) Purify 626 from cell.
- 2) check amount of ~~col-01~~ fragment ~~col-01~~ fragment ~~col-01~~ fragment

Saturday, December 14, 1996.

- 1) Prepare LB, sterile appendages, sterile TE, sterile TE.
- 2) Dissolve 626 prep in sterile TE. Check plasmid with
- 3) Split 293 cells to ~~transformation~~ 626+ ~~transformation~~
- 4) Repeat transformation of ~~old~~ old prep. at ~~different~~ different cells.
- 5) Transform DUSA (new prep) with 42, 45 ~~plasmids~~

Tex, 7c  $\Rightarrow$  10  $\mu$ l of each to 100  $\mu$ l of old DUSA cells  
 42, 45  $\Rightarrow$  0.001  $\mu$ l of each to 100  $\mu$ l of new DUSA cells

Monday, December 16, 1996.

Tex, 7c  $\Rightarrow$  no clones again, efficiency of transform is ~~high~~

What's wrong?  $10^4 \times 10^3 \approx 10^7$  /  $1 \mu$ g pL1  $\Rightarrow \sim 10^8$  /  $\mu$ l ~~plasmid~~  
 Ligation?  
 Not optimal ratio vector/insert (?)  
 pBSK was pBSKS (?)

- 1) Dissolve 626 ~~in~~ in sterile TE, check on EIE amount and quality of the plasmid, check other plasmid as well. (left on)
- 2) Transformation of ~~293~~ with 626+ left on.
- 3) Ligate Tex again, transform DUSA, 5  $\mu$ l vector transform CMV/TTF1. 15  $\mu$ l insert.
- 4) Seed 42, 45 to liquid culture (LB).



22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS

EXHIBIT

D36



PMV - left arm plasma (1.5  $\mu$ l) 7  $\mu$ l (10 $\times$ )  
 p562 - (PONGI ENDCIV) (0.58  $\mu$ l) 100  $\mu$ l (50 $\times$ )

✓ 1.5 ml 2X HES

✓ 1200  $\mu$ l H<sub>2</sub>O

✓ DNA (107  $\mu$ l)

✓ 150  $\mu$ l CaCl<sub>2</sub>

✓ 3 ml  $\rightarrow$  for 6 drops

2X Overlay

(for 100 ml overlay)

50 ml 2X DMEM  
 5 ml 7.5% NaHCO<sub>3</sub>

2 ml FCS

1 ml 100 $\times$  PS

4.3 ml 1.8% Noble agar.

Tuesday, December 17, 1996.

Tex  $\rightarrow$  no clones. Something in fragment preps. interfere with ligation? (It's possible, because of this red. colour strip!)

1) Seed i) CMVITTE, 54 (L2 + ADP) into large scale liquid culture LB.

ii) TIC, 34  $\rightarrow$  small scale liquid culture  $\rightarrow$  WB.

2) Extract 42, 45 with Wizard mini prep, check seed 42, 45 for medium scale prep.

3) Try precipitate fragments 140/SR, 3A SR to purify them.

4) Try partial digestion p223 Bam Nde (Nde - complete, Bam HI - partial dephosphorylate, try cloning with 140 B N B

Try to clean them with Wizard clean up system.

EXHIBIT

D37

tabbly

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



Wednesday, December 18, 1996.

Thursday, December 19, 1996.

Cut pFG140 → (Ban + Nde) (buffer)

→ Sal I + Eco RI (buffer)

Cut 223 with Nde → then with Ban II (partial) →  
(complete) → band → CIP → sector from gel.

Cut 31 with Sal + Eco RI (buffer)

Cut 223 Eco RI (buffer)

31/R, 140/R, 223 Nde, 223 R, 140/Ban Nde

Sunday, December 22, 1996.

- 1) Overlay dishes 293 with neutral red.  
Split 293 cells to dishes → repeat transfection.  
Seed frozen 293 cells for last passage.
- 2) Check 31, 31/Sal, 31/RI, 31, Sal + RI to estimate  
molecular weight of the plasmid, 223/Nde, and partial  
626 - with Ban II.
- 3) Cut fragments from gel, ligate.
- 4) Seed CMV/TTA 59 for large scale liposome culture.
- 5) Seed PK plasmid for WMP.

Sunday, December 23, 1996.

- i) Check CMV/TTA - with Bgl II, Sac I.
- ii) PK? → Hind III + Xba I, Pst I.
- iii) p54 (trap) - Pst I
- iv) 42 - Xba I } 2% agarose  
45 - Xba I

EXHIBIT

D38

- \* 2) Split 293 cells to dishes for transfection.  
 3) Cut 1101/1107 DNA with *EcoRI*.

Transfection:

- 2 dishes pEG100.  
 → 2 dishes E1 + pBHG11  
 → 2 dishes E1 + pBHG11 dEGFP-CMV from ins.

First attempt  
to make KD1  
12/04/96 (7)

500  $\mu$ l 2x HEBES + 450  $\mu$ l H<sub>2</sub>O + 20  $\mu$ l pEG100 + 1 + 20  $\mu$ l BHG11 + 50  $\mu$ l GCL  
 1 + 20  $\mu$ l BHG11 (EGFP-CMV)

Precipitate is coarse  
again where E1 plasmid is used  $\Rightarrow$  purify it (SCE binding)

Saturday, December 4, 1997.

- ↓ 1) Cut 1101/1107 with *EcoRI*.  
 ↓ 2) Split 293 to dishes, cotransfect 1101/1107 with L2  
 ↓ 3) Pick up this ugly things resembling plaques to plate to try to grow up in plaques.  
 ↓ 4) Overlay dishes with NR.

Overlay 2x 200  $\mu$ l

200  $\mu$ l 2x DMEM  
 4  $\mu$ l 2x PS  
 2  $\mu$ l 5% yeast extract.  
 3  $\mu$ l FBS  
 10  $\mu$ l HS  
 2  $\mu$ l 100x t-butylamine  
 20  $\mu$ l bicarb 7.5%.

Transfection. 1101/1107 /RT + control (40)  
 2(x) + L2 (20x)  
 + 54 (20x)

4  $\mu$ l of each transfection mixture

2  $\mu$ l of 2x HEBES.  
 1.8  $\mu$ l of H<sub>2</sub>O  
 DNA  
 + 200  $\mu$ l GCL 2.5M.

EXHIBIT

D39

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



Wednesday, 8, 1996

- 1) Spin left hand plasmid in ~~left~~ gradient 8x H<sub>2</sub>O.
- 2) Split 238 to dishes for transfection.
- 3) Fast 31 with EcoRI → complete digestion and with Sal I → fragment dephosphorylate.
- 4) Cut 225 (21 + SPB prom) / Mde with Bam HI partial → dephosph.
- 5) Fragments 140 Sal + Mde, Bam + Mde → cut from gel.
- 6) Ligate 7ex, 8ex overnight.
- 7) Precipitate 1101/1107 DNA end time, cut with EcoRI.
- 8) Sequence 42.
- 9) Run experiment with TAC gene.

Thursday 9, 1996

- 1) Transform Kurt's left-hand plasmid

Monday, 14, 1996

Transfection 5x LacZ (5μl) + 15x 626 (15μl) (1:3)  
 or 10x LacZ (10μl) + 10x 626 (10μl) (1:1)  
 or 5x LacZ (5μl) + 20x 626 (20μl) (1:4)

Ligation 8ex 225 / Mde + Bam (partial) C, P  
 + 140 / Bam Mde to fragment

Thursday, 14, 1996

- 1) Sequence 42, primers (21) 4, (21) 5 →

→ sequence → 109, 1101

→ No sequence at all!  
 What is it?

EXHIBIT

D40

8 pl premix  
 3 pl p52  
 7 pl primer (4 or 5)  
 8 pl H<sub>2</sub>O

circ. lig

Monday, 20, 1996.

8 ex ( p223 / complete hydr. Nde + Partial Bam )  
 ( + B fragment pFG100 / Bam + Nde )



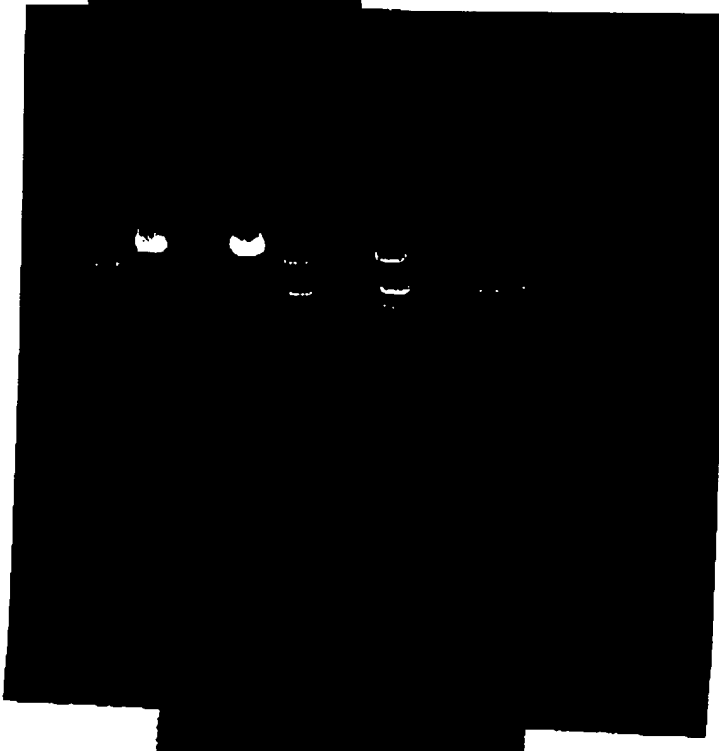
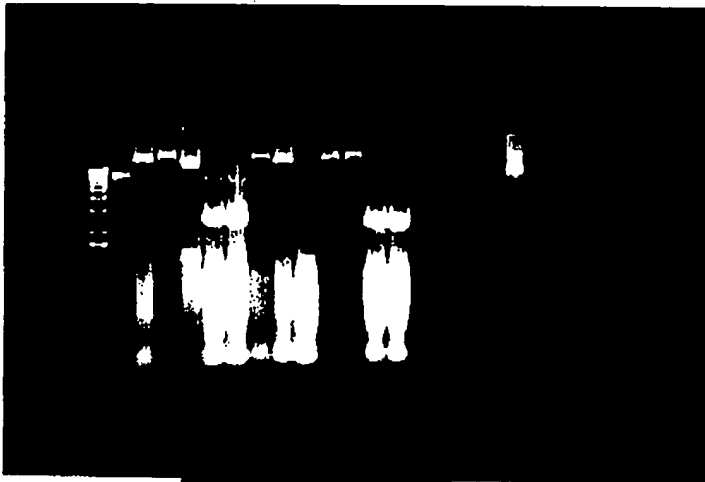
Miniprep 5: 8 ex (1-12).

1) Ladder.

2) p223.

3) →

clones 8 ex 1-12.



1) Ladder

2) pFG100 / Bam + Nde

3) p223 Bam + Nde.

(upper fragment -  
 because of incomplete  
 Nde digestion)

4) 81

5) 82

6) 86

7) 87

8) 88

9) 89

10) 810

clones 82, 86, 87,

Nde +  
 + Bam

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-144 200 SHEETS



EXHIBIT

D41

tabbles

Cloning 9ex

82 / ~~Not~~ XbaI - vector 1 fragment  
 ~ 10 kbp  
 1 fragment ~ 9 kbp

insert p31 / EcoRI + XbaI -- 1 fragment

~~from PCR every 1 Not XbaI XbaI~~  
~~but Not XbaI XbaI~~

Ligation

vector

82 / R1 XbaI 1 fr

5 µl (0.1 / 10)

insert

31 / R1 XbaI 1 fr

5 µl (0.1 / 15)

2 µl 1/20

2 µl Buffer

1 µl Ligase

2x080

prep. of 82

1) ladder

2) 240

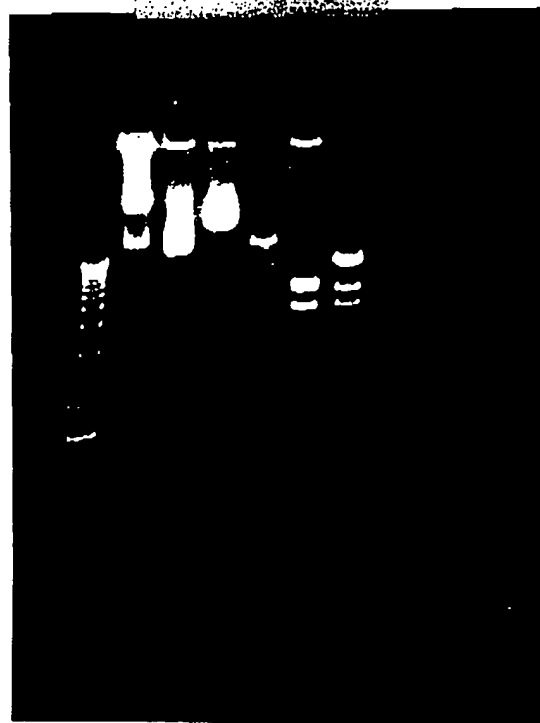
3) 223

4) 82

5) 110

6) 225 / Ban + Not

7) 80



EXHIBIT

D42

tabbies

January 25, 1997.

g c and gex  $\rightarrow$  ~100 bad colonies / dish  
in both cages  $\Rightarrow$  (defective) <sup>vector</sup>Transfection: 1101/1107/EORI (0.5 r/ $\mu$ l)  
+ pK82 (E4  $\rightarrow$  SPB) (10 r/ $\mu$ l)  
+ CMV/TTF (2 r/ $\mu$ l)

10 6cm dishes: 4 control + 6 experiment

- 1) control A) 8  $\mu$ l (4 r) 1101/1107 (1 ml precip.)  
2) control B) 8  $\mu$ l 1101/1107/EORI + 5  $\mu$ l CMV/TTF  $\Rightarrow$  (4 r) (10 r)  
 $\Rightarrow$  control on wt and toxicity of TTF.

3) exp. 8  $\mu$ l (1101/1107/EORI)  $\Rightarrow$  4 rA) + 10  $\mu$ l p82  $\Rightarrow$  10 r  
+ 3  $\mu$ l CMV/TTF  $\Rightarrow$  6 rB) 8  $\mu$ l 1101/1107/EORI  $\Rightarrow$  4 r  
+ 50  $\mu$ l p82  $\Rightarrow$  5 r  
+ 5  $\mu$ l CMV/TTF  $\Rightarrow$  10 rC) 8  $\mu$ l (1101/1107/EORI)  $\Rightarrow$  4 r  
+ 15  $\mu$ l p82  $\Rightarrow$  15 r  
+ 1  $\mu$ l CMV/TTF  $\Rightarrow$  2 r22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

tabler

D43

Sunday, February 2, 1987

Transfection

8 large dishes.

2 - 626 + 602 (left-hand)

2 - 41 + 602 (left-hand)

2 - 1101/1107/RT - control + CMV/ITC

2 - 1101/1107/RT + p82 + CMV/ITC

400 DNA for 2 ml of precipitate - each dish

(626) 4 ml of precipitate  $\Rightarrow$

$\Rightarrow$  2 ml NEBS 2X + 100  $\mu$ l  $\text{CaCl}_2$

p602 = 80  $\mu$ l (40x)  $\checkmark$  2 ml  $\text{H}_2\text{O}$

p602 = 40  $\mu$ l (40x)  $\checkmark$

(41)

p41 = 40  $\mu$ l (40x)  $\checkmark$

p602 = 40  $\mu$ l (40x)  $\checkmark$

(82 - control)  $\checkmark$  1101/1107/RT - 25  $\mu$ l (12x)  
 $\checkmark$  pCMV/ITC - 20  $\mu$ l (40x)

(82 - exper.)  $\checkmark$  1101/1107/RT - 25  $\mu$ l (12x)  
 $\checkmark$  pCMV/ITC - 20  $\mu$ l (40x)  
 $\checkmark$  p82 - 30  $\mu$ l (40x)

EXHIBIT

tabbies

D44



Wednesday, February 5, 1997

Transfection of 231 with 626 + LacZ (Mohan's with ITR)  
~~626~~ + LacZ (--- u ---)

626 + LacZ (Good's pMVS4...)

(626 + M)

2 ml HEB5 2X + 2 ml H<sub>2</sub>O + 100  $\mu$ l G418

✓ p626 - 100  $\mu$ l (500)

✓ pMohan's - 5  $\mu$ l (80)

(626 + M)

✓ pDUG11 - 50  $\mu$ l (500)

✓ pMohan's - 5  $\mu$ l (80)

(626 + C)

✓ p626 - 100  $\mu$ l (500)

✓ pMVS4 - 20  $\mu$ l (200)

TTC TCTAGA AGT.... ATGA GATCTAGA

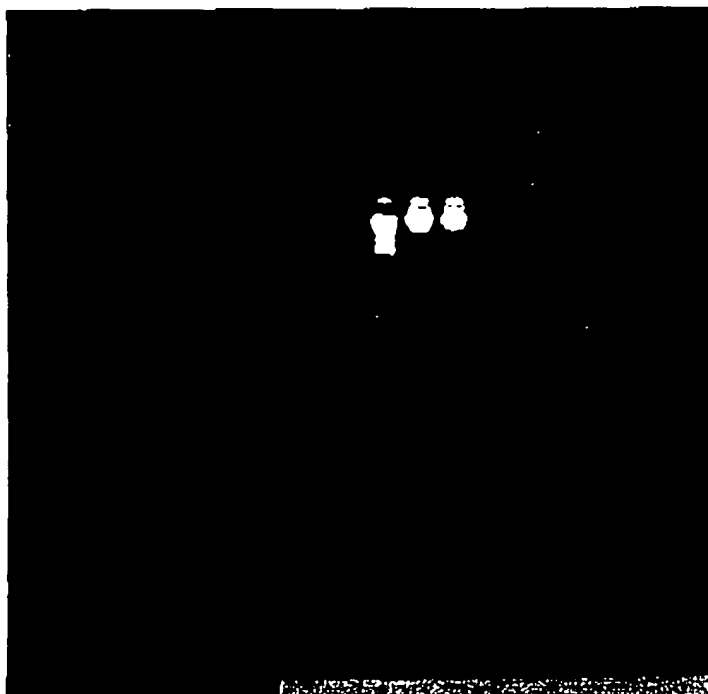
1) ladder

2) - 42 / X6

3) - 45 / X6

⇒ linear form

2nd X6 in the  
 plasmid is methylated!



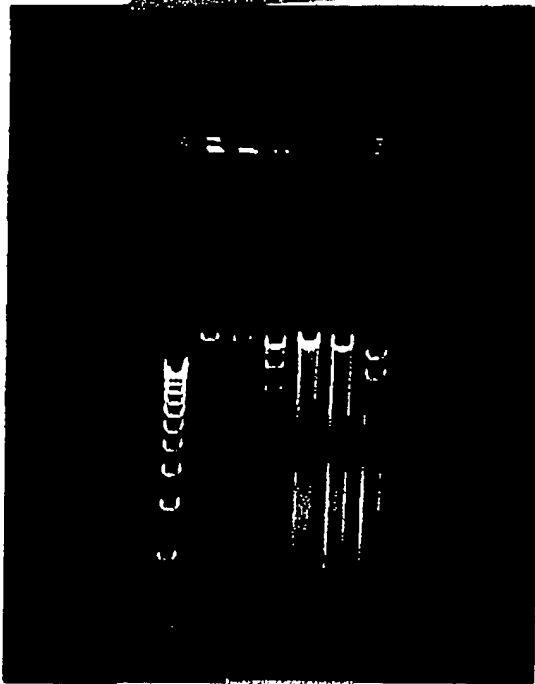
EXHIBIT

D45

tabbler



Sunday, February 9, 1997



Analysis of 1101/1102 (R1)  
 54  
 plagues 54-2

1) Ladder

2) 1101/1102 Psc I  
 3) 1101/1102 Psc I + Bam HI  
 4) 1101/1102 Psc I + Bam HI

5) 54-2

6) 54-2 Psc

7) 54-2 Psc + Bam HI

Conclusion 54-2 - wt  
 but there are  
 additional bands

PCR primers  
 (D1, K1) 5'

1) Ladder

2) pFG140

3) p82

4) p54

5) plagues 54-2

6) plagues 54-2

7) plagues 54-2

8) plagues 54-2

9) plagues 54-2

10) plagues 54-2

11) plagues 54-2

12) plagues 54-2

13) plagues 54-2

14) plagues 54-2

15) plagues 54-2

16) plagues 54-2

17) plagues 54-2

18) plagues 54-2

19) plagues 54-2

20) plagues 54-2

21) plagues 54-2

22) plagues 54-2

23) plagues 54-2

EXHIBIT

D46

February 10, 1997.

Transfection of Mohr's 293/CNV/TTE with p82.

**Control** : 50  $\mu$ l of 1101/1107/EcoRI (10x)  
 + 20  $\mu$ l pCMT/TTE (20x)  
 + 100  $\mu$ l p82 (100x)

3 ml H<sub>2</sub>O  
 3 ml HEBS 2X  
 300  $\mu$ l CaCl<sub>2</sub> 2.5M.

**p82** 50  $\mu$ l 1101/1107/R1.  
 + 20  $\mu$ l pCMT/TTE  
 + 100  $\mu$ l carrier DNA

February 11, 1997.

Repetition of ex. vector  $\rightarrow$  CIP - heat inactivation

92/R1-K6 (vector) - 5  $\mu$ l -  
 51/R1-K6 (insert) - 10  $\mu$ l  
 Lig. buffer - 2  $\mu$ l  
 Ligase - 1  $\mu$ l.

February 14, 1997.

Transfection with 1101/1102/EcoRI + or pL2  
 p54 (L2-MP)

Cont A	10 $\mu$ l 1101/1102/EcoRI (5x)	+ nothing
Cont B	-----	+ 30 $\mu$ l carrier (30x)
L2 A	-----	+ 50 $\mu$ l L2 (30x)
L2 B	-----	+ 30 $\mu$ l L2 (30x)
54 A	-----	+ 50 $\mu$ l 54 (25x)
54 B	-----	+ 50 $\mu$ l 54 (25x)

1 ml HEBS 2X + 1 ml H<sub>2</sub>O + 100  $\mu$ l CaCl<sub>2</sub>

EXHIBIT

tabler

D47

Cloning experiment 9 (9x)

with two vectors 82 / Vekt + R1 (1)

82 / R1 + (68) (2)

C Vekt 0.1 / 5 site ~ 10?

C Insert 0.1 / 5 ~ 2?

9ex 1 - Spl Vekt + Spl Insert

9ex 2 - Spl Vekt + Spl Insert

February 20, Thursday

Analysis of  
restriction  
pattern of  
plasmids

1) Redder 1st

2) 1107 / Pac 1 + Bam HI

3) 1107

4) 54-1

5) 54-2

6) 54-3

7) 54-4

8) 54-5

Pac 1 +  
Bam HI

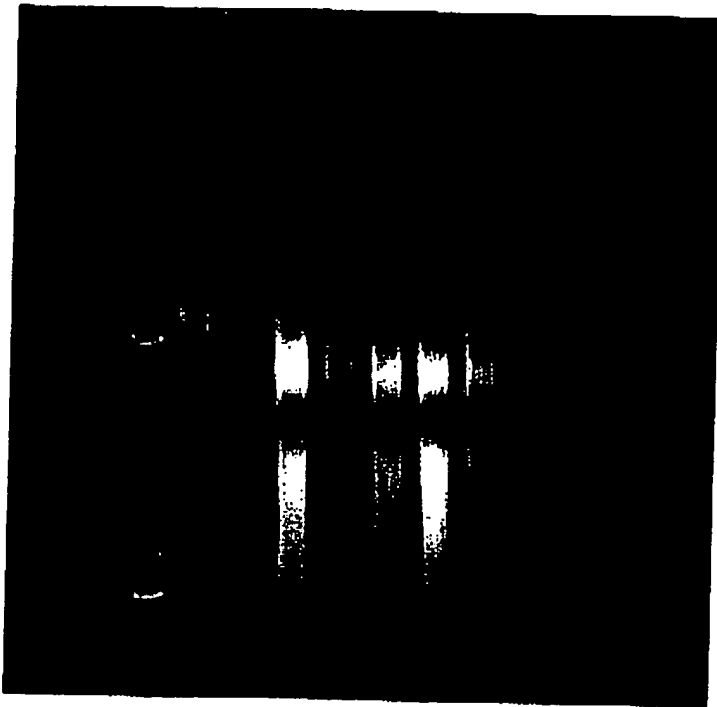
for 54-1

54-3

54-4

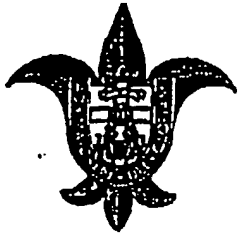
restriction pattern

is as expected for  
recombinant virus



EXHIBIT

D48



**SAINT LOUIS  
UNIVERSITY**

1402 South Grand Blvd.  
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Phone: 814-577-8432  
FAX: 814-773-8403

Department of Molecular  
Microbiology & Immunology

Health Sciences Center  
School of Medicine

**Facsimile Transmittal**

Date: 12 - 3 - 02

From: William Wold

Phone: 314-577-8432

Fax: 314-773-3403

Deliver to: Dan Kasten

Phone: Fax: 552-76305

Total number of pages including this page: 12

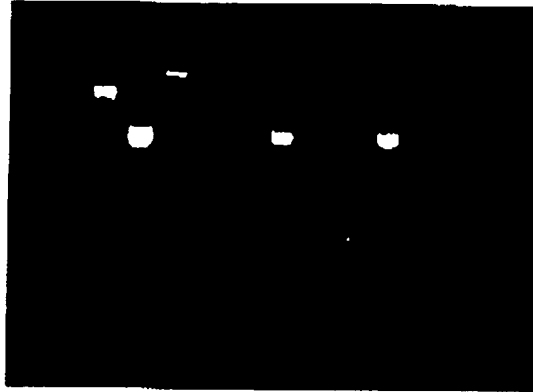
Message:

Kostya's lab notes.

KO1 is made  
2/20/97

2-20-97

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



PCR of Wnt prep  
from previous picture  
primers (2) 1 and (2) 7

- 1) Ladder 1ul
- 2) pAG140 (dl 309)
- 3) pS4
- 4) Mock
- 5 - 9) -
- S4 1 - 5
- respectively

viruses S4-2  
and S4-5  
contain both wt  
and recombinant AGP.

there is band as is wt in mock!

S4-1  
S4-3  
S4-4 } - recombinants!

02 - 25 - 97

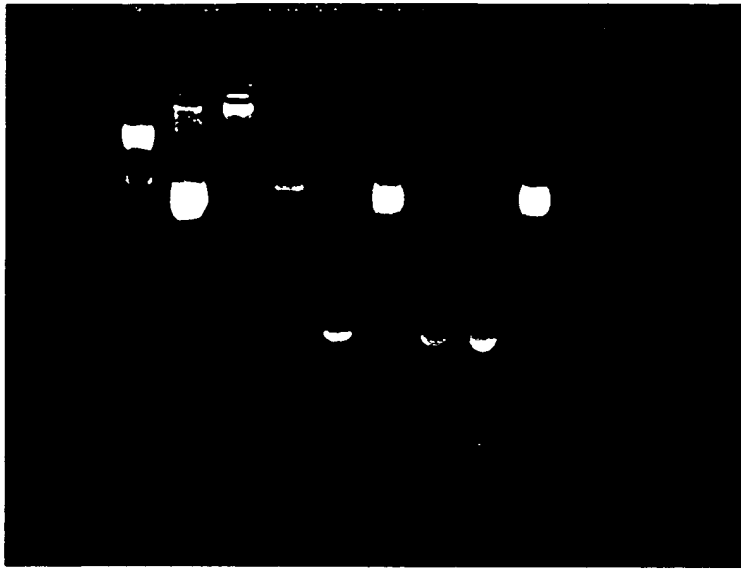
get. → 2 time purified from gel vector.  
p82 / R1 & C. } A fr.  
insert p31 / R1 & C.

vector 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer

vector lig 10ul 82 + 8ul H<sub>2</sub>O + 2ul buffer + 4ul ligase

vector + insert 10ul P2 + 8ul insert + 2ul buffer + 1ul ligase

2-20-91



PCR of Unit frags  
from previous picture.  
primers 10D1 and 10D7.

- 1) Gadder 1ul
- 2) pAC140 (dl 505)
- 3) p54
- 4) Hock
- 5 - 9) -
- 54 1 - 5
- respectively.

viruses 54-2  
and 54-5  
contain both wt  
and recombinant ADP!

there is band as in wt in movie!

54-1  
54-3  
54-4 } - recombinants!

52-25-97

Den. → 2 time purified from gel vector.  
p52 / R1 Xba } 11 fr.  
insert p31 / R1 Xba

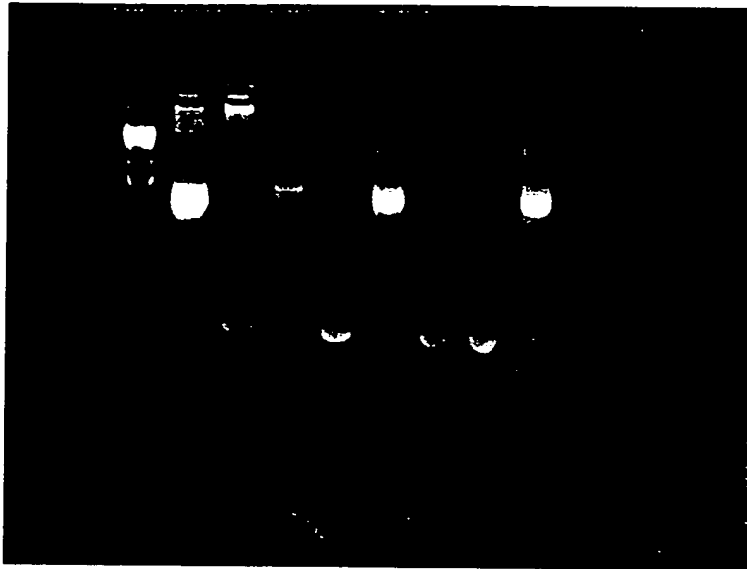
vector 10  $\mu$ l 82 + 8  $\mu$ l H<sub>2</sub>O + 2  $\mu$ l buffer

vector only 10  $\mu$ l 82 + 8  $\mu$ l H<sub>2</sub>O + 2  $\mu$ l buffer + 1  $\mu$ l ligase

vector + insert 10  $\mu$ l 82 + 8  $\mu$ l insert + 2  $\mu$ l buffer + 1  $\mu$ l ligase  
in 100  $\mu$ l

2-20-91

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



PCR of lint frags  
from previous picture.  
primers led 1 and led 7.

- 1) ladder 1ul.
- 2) pAG140 (dl 309)
- 3) pS4
- 4) mock
- 5-9) -  
- S4 1-5  
respectively.

viruses S4-2  
and S4-5  
contain both wt  
and recombinant ADP.

there is band as in wt in movie!

S4-1 }  
S4-3 } - recombinants!  
S4-4 }

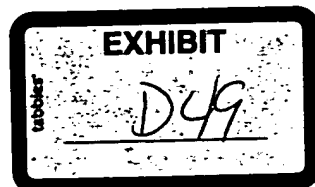
S2-25-97

Dec. → 2 time purified then gel vector.  
p82 / R1 X62.  
insert p31 / R1 X62 } 11 fr.

vector 10  $\mu$ l 82 + 8  $\mu$ l H<sub>2</sub>O + 2  $\mu$ l buffer

vector + lig 10  $\mu$ l 82 + 8  $\mu$ l H<sub>2</sub>O + 2  $\mu$ l buffer + 7  $\mu$ l ligase

vector + insert 10  $\mu$ l 82 + 8  $\mu$ l insert + 2  $\mu$ l buffer + 1  $\mu$ l ligase  
~~in 100  $\mu$ l~~





82-25-97

54-10 plaques - plaque type that I like

WT + Fec or Fec with high MOI?

Sex - try 82-16?

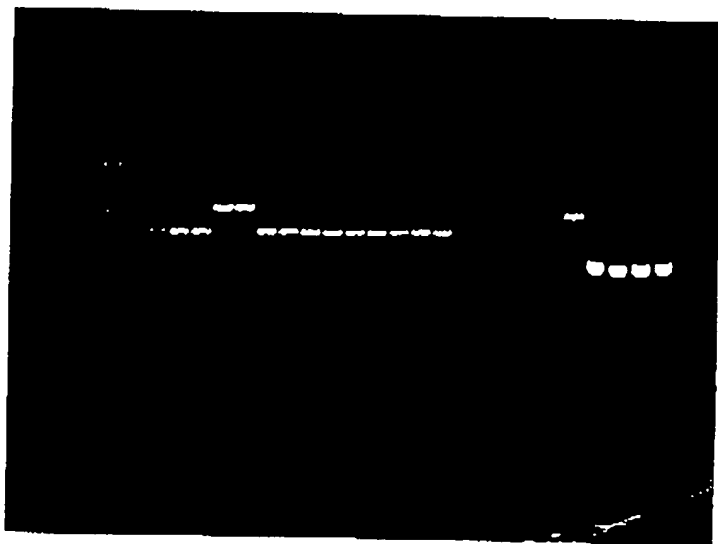
03-05-97 Wednesday

Clay Experiment 10 ex  $\Rightarrow$  pL2 + E3 from gp13K (p31)  
(sites EcoRI + NdeI).11 ex  $\Rightarrow$  pL2 + E3 from dPE3X66 + ADP  
(p42)  
(sites  $\Rightarrow$  EcoRI + NdeI)

EXHIBIT

tabbies

D50

PCR analysis  
of plaques p82  
and p54

1) Ladder

2) Blank

3) mock

4) 1101/1107

5) pF6140

6) p225

7) p82

8) WT 1101/1107

9) 821

10) 823

11) 824

12) 826

13) 828

14) 829

15) 8210

16) 8211

17) 8212

18) 8213

19) 8214

20) 8215

21) 8216

22) 8217

23) 8218

24) 8219

25) 8220

26) 8221

primers

ND 10, 11, 12

12) - Ladder

13) - Blank

14) - mock

15) 1101/1107

16) pF6140

17) p225

18) p82

19) WT 1101/1107

20) 821

22) 54

23) 546

24) 548

25) 549

26) 5410

27) 5411

28) 5412

29) 5413

30) 5414

31) 5415

32) 5416

33) 5417

34) 5418

35) 5419

36) 5420

37) 5421

38) 5422

39) 5423

40) 5424

41) 5425

42) 5426

43) 5427

44) 5428

45) 5429

46) 5430

47) 5431

48) 5432

49) 5433

50) 5434

51) 5435

52) 5436

53) 5437

54) 5438

55) 5439

56) 5440

57) 5441

58) 5442

59) 5443

60) 5444

61) 5445

62) 5446

63) 5447

64) 5448

65) 5449

66) 5450

67) 5451

68) 5452

69) 5453

70) 5454

71) 5455

72) 5456

73) 5457

74) 5458

75) 5459

76) 5460

77) 5461

78) 5462

79) 5463

80) 5464

81) 5465

82) 5466

83) 5467

84) 5468

85) 5469

86) 5470

87) 5471

88) 5472

89) 5473

90) 5474

91) 5475

92) 5476

93) 5477

94) 5478

95) 5479

96) 5480

97) 5481

98) 5482

99) 5483

100) 5484

101) 5485

102) 5486

103) 5487

104) 5488

105) 5489

106) 5490

107) 5491

108) 5492

109) 5493

110) 5494

111) 5495

112) 5496

113) 5497

114) 5498

115) 5499

116) 5500

117) 5501

118) 5502

119) 5503

120) 5504

121) 5505

122) 5506

123) 5507

124) 5508

125) 5509

126) 5510

127) 5511

128) 5512

129) 5513

130) 5514

131) 5515

132) 5516

133) 5517

134) 5518

135) 5519

136) 5520

137) 5521

138) 5522

139) 5523

140) 5524

141) 5525

142) 5526

143) 5527

144) 5528

145) 5529

146) 5530

147) 5531

148) 5532

149) 5533

150) 5534

151) 5535

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154) 5538

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156) 5540

157) 5541

158) 5542

159) 5543

160) 5544

161) 5545

162) 5546

163) 5547

164) 5548

165) 5549

166) 5550

167) 5551

168) 5552

169) 5553

170) 5554

171) 5555

172) 5556

173) 5557

174) 5558

175) 5559

176) 5560

177) 5561

178) 5562

179) 5563

180) 5564

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182) 5566

183) 5567

184) 5568

185) 5569

186) 5570

187) 5571

188) 5572

189) 5573

190) 5574

191) 5575

192) 5576

193) 5577

194) 5578

195) 5579

196) 5580

197) 5581

198) 5582

199) 5583

200) 5584

201) 5585

202) 5586

203) 5587

204) 5588

205) 5589

206) 5590

207) 5591

208) 5592

209) 5593

210) 5594

211) 5595

212) 5596

213) 5597

214) 5598

215) 5599

216) 5600

217) 5601

218) 5602

219) 5603

220) 5604

221) 5605

222) 5606

223) 5607

224) 5608

225) 5609

226) 5610

227) 5611

228) 5612

229) 5613

230) 5614

231) 5615

232) 5616

233) 5617

234) 5618

235) 5619

236) 5620

237) 5621

238) 5622

239) 5623

240) 5624

241) 5625

242) 5626

243) 5627

244) 5628

245) 5629

246) 5630

247) 5631

248) 5632

249) 5633

250) 5634

251) 5635

252) 5636

253) 5637

254) 5638

255) 5639

256) 5640

257) 5641

258) 5642

259) 5643

260) 5644

261) 5645

262) 5646

263) 5647

264) 5648

265) 5649

266) 5650

267) 5651

268) 5652

269) 5653

270) 5654

271) 5655

272) 5656

273) 5657

27

02-25-01

p82 + 1101/1107 / Ecol / plaques!

AC wt 1 - medium size } wt control  
AC wt 2 - large size }transfection  
from 01-25

A 1, (2) - medium

B 3 - medium

➤ 4 - small

5 - medium

B 6, 7, 8 - medium

➤ 9 - small

C - 10 - medium

➤ (11) - small

(12, 13, 14) - medium

(15, 16, 17) - medium } plaques?  
or not.transfection  
from 02-02↓  
Prophal contamination in plate wells827 ➤ lost due to Prologe of Eppendorf  
and 547 tubes.⇒ no recombinants with p82 according to PCR results.  
p54 = ? ⇒ no product of PCR, just RNA.

EXHIBIT

tabbles

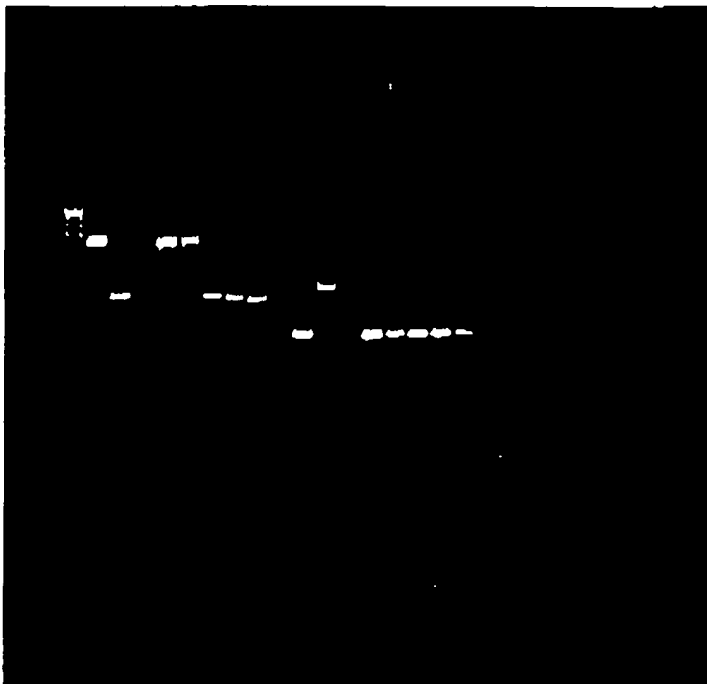
DST

05-06-97

Ligation 10 ex, 11 ex

5  $\mu$ l vector (L2/ENAH)  $\rightarrow$  V+ ligase  $\rightarrow$  VL+ 10  $\mu$ l 3' ENL  $\rightarrow$  10 ex L+ 15  $\mu$ l 3' ENH  $\rightarrow$  10 ex H+ 15  $\mu$ l 4' EN  $\rightarrow$  11 ex

PCR Analysis of plaques



1) ladder

2) 100

3) 5' H

4) mock

5) WT 1101/1102

6) 546

7) 548

8) 540

9) 5400

10) blank

11) pEG100

12) p82

13) mock

14) WT 1101/1102

15) 822

16) 825

17) 827

18) 829

548, 549, 5410

- recombinants

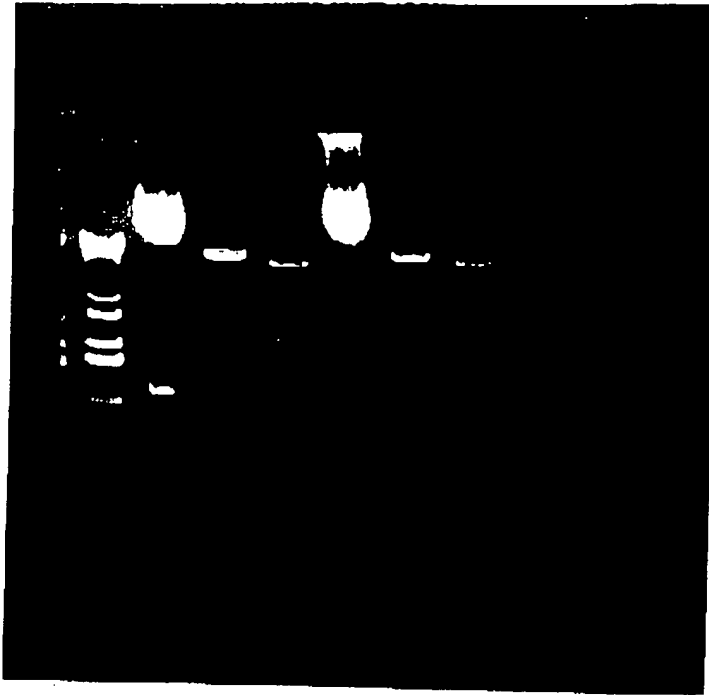
with p82 all  
plaques are WT

EXHIBIT

tabbles

DSQ

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



- 1) Ladder
  - 2) pL2
  - 3) p31
  - 4) p42
  - 5) pL2
  - 6) p31
  - 7) p42
- { EcoRI +  
 Nde,  
 buffer MC  
 { EcoRI +  
 + Nde,  
 buffer )

03-07-97

Transfection p82 into 293/TTF.

12 dishes — 6 - control ( 3 ml of precip. ) — 60% DNA  
 6 - experiment ( — 1 — ) — 1 —

(control) 25 µl 1101/1107/R1 (5r) 1.5 ml LIEBS 2X  
 + 5 µl pCMV TTR (10r) 1.5 ml H<sub>2</sub>O  
 + 30 µl GoldTiter. (~300) 150 µl CaCl<sub>2</sub>  
 DNA.

(expt.  
 p82) 25 µl viral DNA.  
 + 5 µl pCMV TTR  
 + 30 µl p82

EXHIBIT

tabbies

D53

03-10-97

Cloning 10 ex, 11 ex.

1 - ladder

2 - p12 7

3 - p31

4 - p42

5 - 10L-1

6 - 10L-2

7 - 10L-3

8 - 10L-4

9 - 10L-5

10 - 10L-6

11 - 10L-7

12 - 10H-1

13 - 10H-2

14 - 10H-4

15 - 10H-5

16 - 10H-6

17 - 10H-7

18 - 10H-8

19 - 11-1

20 - 11-2

21 - 11-3

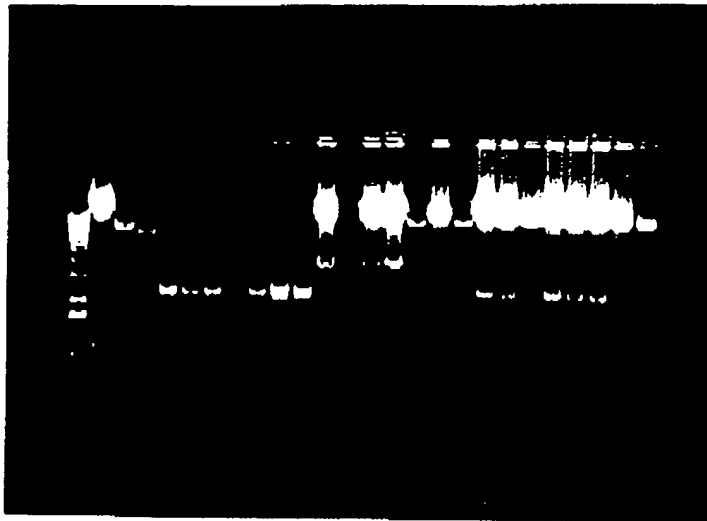
22 - 11-4

23 - 11-5

24 - 11-6

25 - 11-7

26 - 11-8



12 - all clones - rat 11

10H - all clones - recombinant  
lower band was visible in  
all clones in short runtake 11 and 10  
for GSC prep.

Enoki + Mest

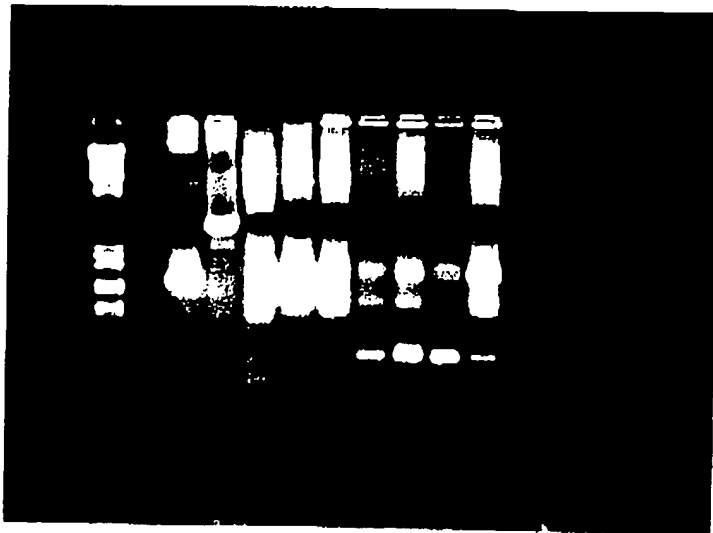
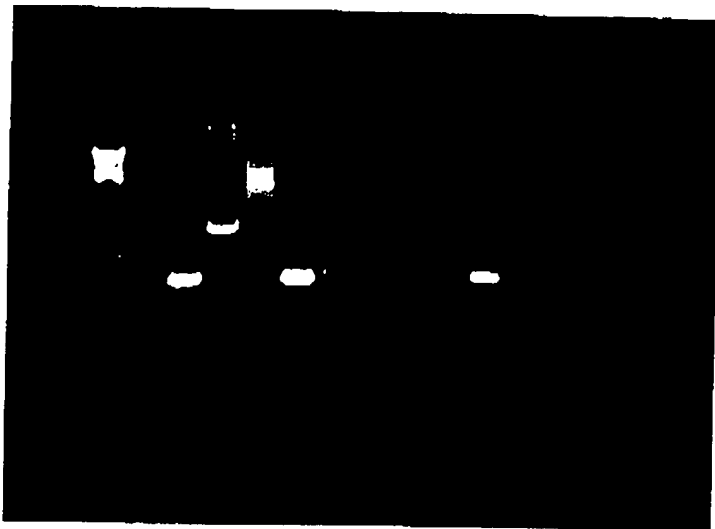
EXHIBIT

tabbies

DS7

03-12-97

lint traps of small amount (plate) of 293/TTC  
infected with slow growing pinases p82(?)  
PCR, primers 10, 11.



- 1) ladder.
- 2) blank (H<sub>2</sub>O)
- 3) p82.140.
- 4) p82
- 5) mock.
- 6) wt.
- 7) 8212
- 8) 8214
- 9) 8215
- 10) 8216
- 11) 82-17.

Conclusions:

blank. clean.

in 82-12, 82-17 -

band, corresponding to wt.

and weak additional

band corresp. to p82.

(does not present in wt!!!)

something of a bit smaller  
MW present in mock

50 SHEETS  
22-141  
22-142 100 SHEETS  
22-143 200 SHEETS



EXHIBIT

DSS

3-17-97

PCR analysis of p82 resulting plaques. primers

WD 8, WD 9 -

- SPB - promoter  
specific.

1) ladder.

2) blank.

3) pG140.

4) p82

5) mock.

6) wt (10<sup>4</sup>/10<sup>7</sup>)

7) 821

8) 822

9) 823

10) 824

11) 825

12) 826

13) 827

14) 828

15) 829

16) 8210

17) 8211

18) 8212

19) 8214

20) 8215

21) 8216

22) 8217

On check, there is SPB in 293 cells,  
viruses are rather wt than  
recombinants, need to use

(10) 8, (11) combination of primers / 10 - SPB specific.

3-18-97

Sequencing

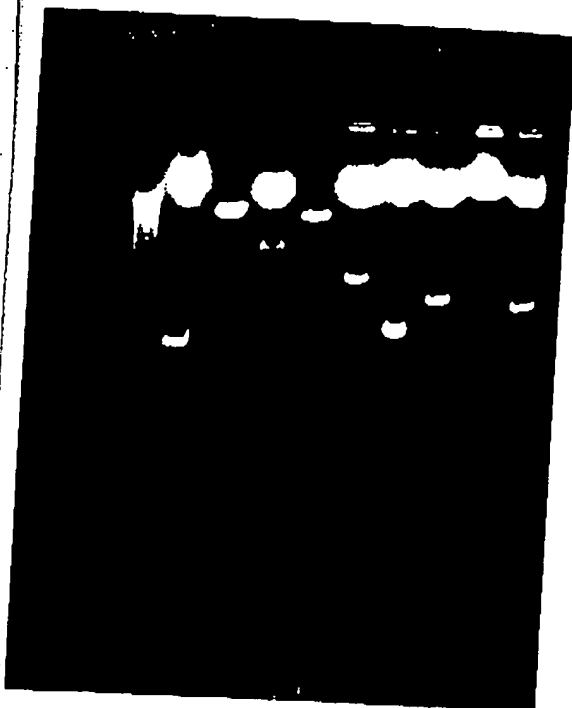
KD9 ( p21, primer KD10)

KD10 ( p21, primer KD11)

KD11 ( p111, primer KD4)

KD12 ( p111, primer KD5)

Restriction pattern of C5cl preps. of p101, p111



- 1) 1 kb ladder
- 2) L2 / R1 + Nde1
- 3) p31 / R1 + Nde1
- 4) p101 / R1 + Nde1
- 5) p42 / R1 + Nde1
- 6) p111 / R1 + Nde1
- 7) L2 / Pac1 + Spe1
- 8) p101 / Pac1 + Spe1
- 9) L2 / Spe1 + Xba1
- 10) p111 / Spe1 + Xba1

3-21-97

Analysis of sequences KD9, 10, 11, 12.

Everything is fine with p111. As predicted.

p21 - sequence with all primer is fine.

sequence with KD10 primed shows that the  
 E. coli sequence is going after Ad nt 35858 and  
 up to the end of sequence (approx 600 kb) → see

EXHIBIT

DS7

tabbler





⇒ p426 and p82 are not responsible for virus.  
Need to check p34611 for the same mutation.

EXHIBIT

DS8

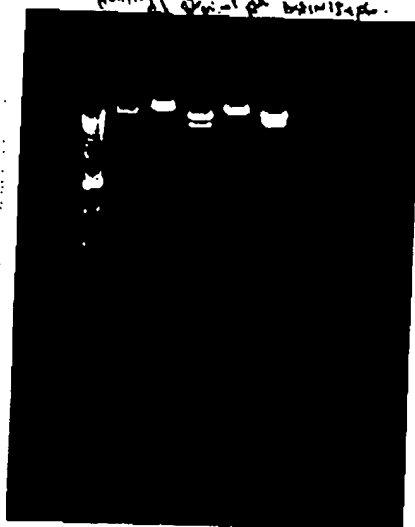
tabler

12/27/97  
3/27/97

3-20-97 Regues. (experiments of 02/05-02/02)  
626M 1-12 (12)  
626c 1-6 (6)  
11M 1-4 (4)

3-25-97

22-141 30 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS  
22-141 30 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1) Colider 148

Buffer-Mc.

2) 1101/1107 DNA / EcoRI + SpeI

3) p82

4) p82 / Bst 1107 I

5) p82 / XbaI

6) p82 / Bst 1107 I + XbaI

There is Bst 1107 I site at Ad 23012!

2nd is after SPB promoter

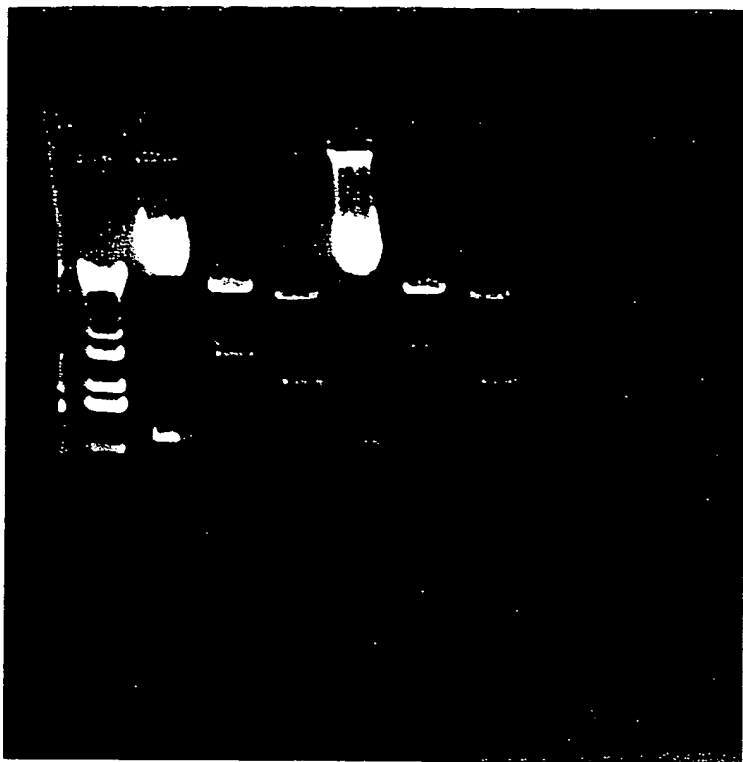
3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI  
8X Gcn dishes.

3 dishes 101 (for 1 dish) 250  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 101  
3 dishes 111 (for 1 dish) 250  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O + 10  $\mu$ l 111  
2 dishes control (for 1 dish) 250  $\mu$ l NEBS 2X + 200  $\mu$ l H<sub>2</sub>O  
+ 10  $\mu$ l 1101/1107 + 25  $\mu$ l GCl<sub>2</sub>.

EXHIBIT

D59



- 1) ladder
  - 2) pL2
  - 3) p31
  - 4) p42
  - 5) pL2
  - 6) p31
  - 7) p42
- { EcoRI +  
 Nde,  
 buffer ME  
 { EcoRI +  
 + Nde,  
 buffer D

03-07-97

Transfection p82 into 293/TTR.

12 dishes — 6 - control (3ml of precip) — 60% DMG  
 6 - experiment ( — — — — — ) — — — — —

(control) 25µl 1101/1107/R1 (5x) 1.5ml LEBS 2X  
 + 5µl pCMV TTR (10x) 1.5ml H<sub>2</sub>O.  
 + 30µl carrier DNA. (~30x) 150µl CaCl<sub>2</sub>

(cover  
 p82) 25µl viral DMG.  
 + 5µl pCMV TTR  
 + 30µl p82

EXHIBIT

tabbler

D60

03410-97

Cloning 10 ex 41 ex

1 - ladder

2 - pL2 7

3 - pB1

4 - p42

5 - 10L-1

6 - 10L-2

7 - 10L-3

8 - 10L-4

9 - 10L-5

10 - 10L-6

11 - 10L-7

12 - 10H-1

13 - 10H-2

14 - 10H-4

15 - 10H-5

16 - 10H-6

17 - 10H-7

18 - 10H-8

19 - 11-1

20 - 11-2

21 - 11-3

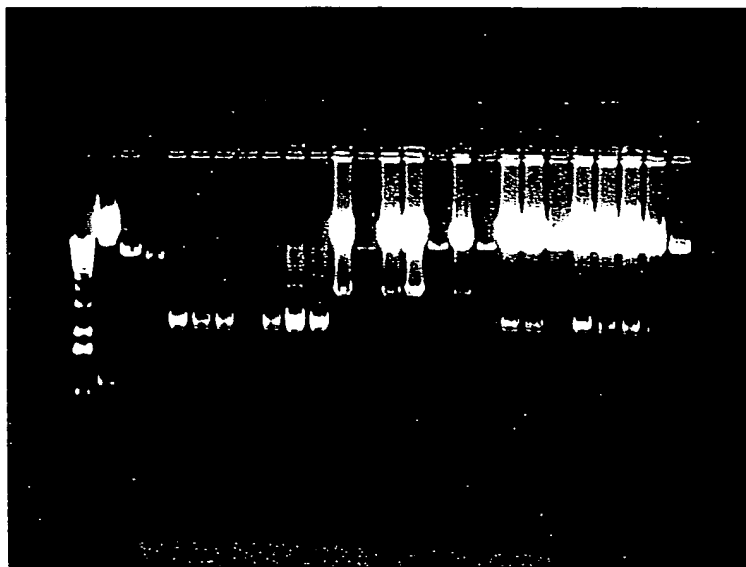
22 - 11-4

23 - 11-5

24 - 11-6

25 - 11-7

26 - 11-8



19 - all clones - rect's

10H - all clones - recombinant  
lower band was visible in  
all clones in short run

take 11 and 101  
for C5C6 maps.

Enob1 + Nde1

EXHIBIT

D61

03-12-97

first preps of small amount (plate) of 293/TTF  
infected with slow growing viruses p82(?).  
PCR, primers 10, 11.

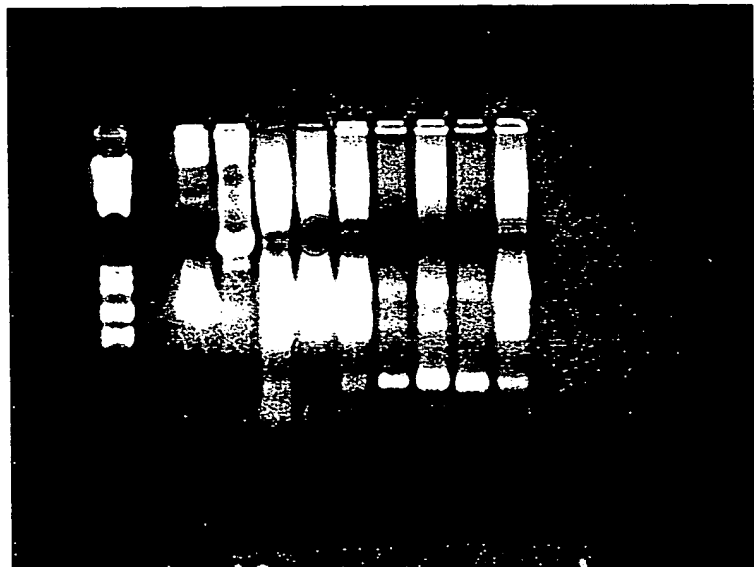
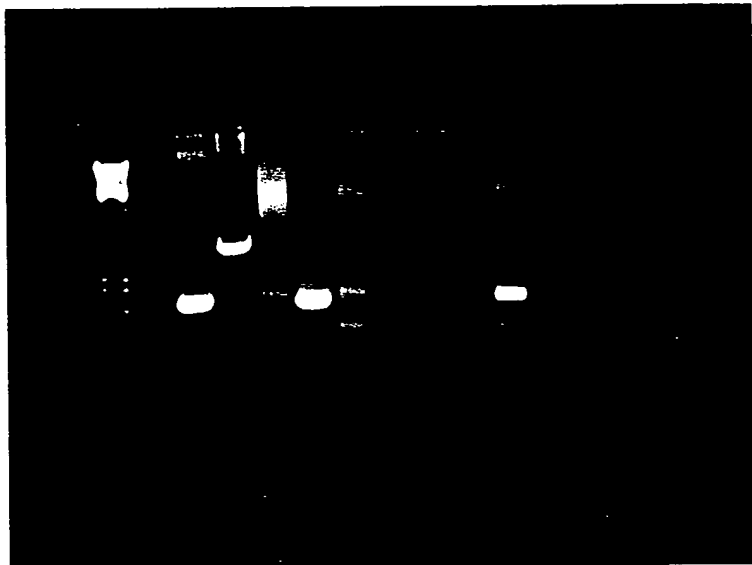
- 1) ladder.
- 2) Blank (H<sub>2</sub>O)
- 3) pF6140.
- 4) p82
- 5) Mock.
- 6) wt.
- 7) 82-12
- 8) 82-14
- 9) 82-15
- 10) 82-16
- 11) 82-17.

Conclusions:

Blank - clean.

in 82-12, 82-17 -  
band, corresponding to wt.  
and weak additional  
band corresp. to rec.

(does not present in mock!!!  
something of a bit smaller  
than present in mock



EXHIBIT

tabbies

D62

3-17-97

PCR analysis of p82 resulting plaques (primers

wd 8, wd 9 -

- SPB - promoter -  
specific).

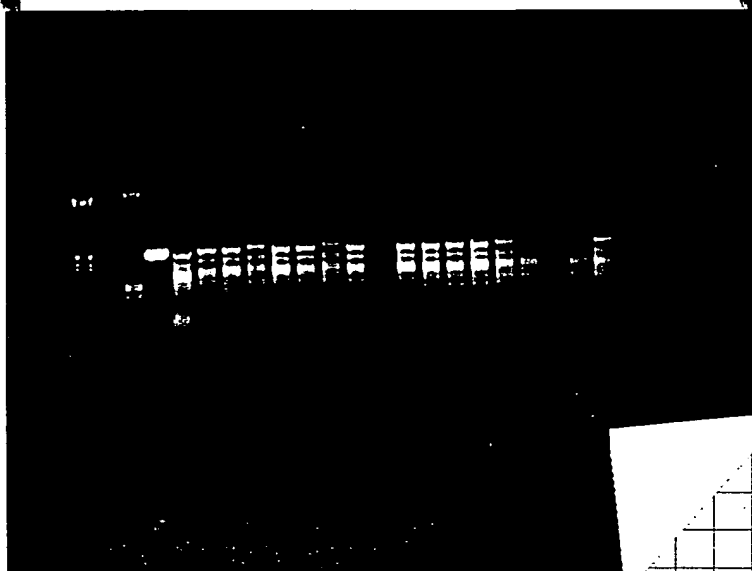
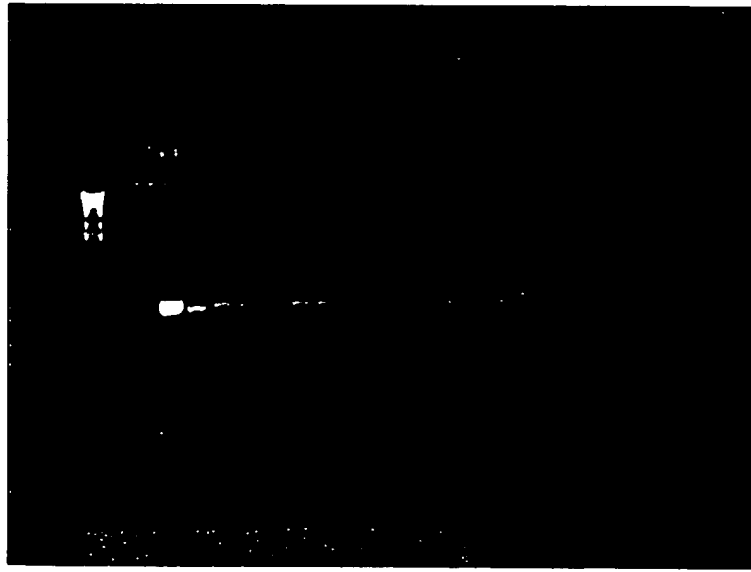


EXHIBIT  
D63



- 1) ladder.
- 2) blank.
- 3) pF6140.
- 4) p82
- 5) mock.
- 6) wt 1101/1107
- 7) 821
- 8) 822
- 9) 823
- 10) 824
- 11) 825
- 12) 826
- 13) 827
- 14) 828
- 15) 829
- 16) 8210
- 17) 8211
- 18) 8212
- 19) 8214
- 20) 8215
- 21) 8216
- 22) 8217

Conclusion: there is SPB in 293 cells,  
viruses are rather wt than  
recombinants, need to use

(10) 8, (11) combination of primers (9 - SPB specific)  
(11 - AI specific)

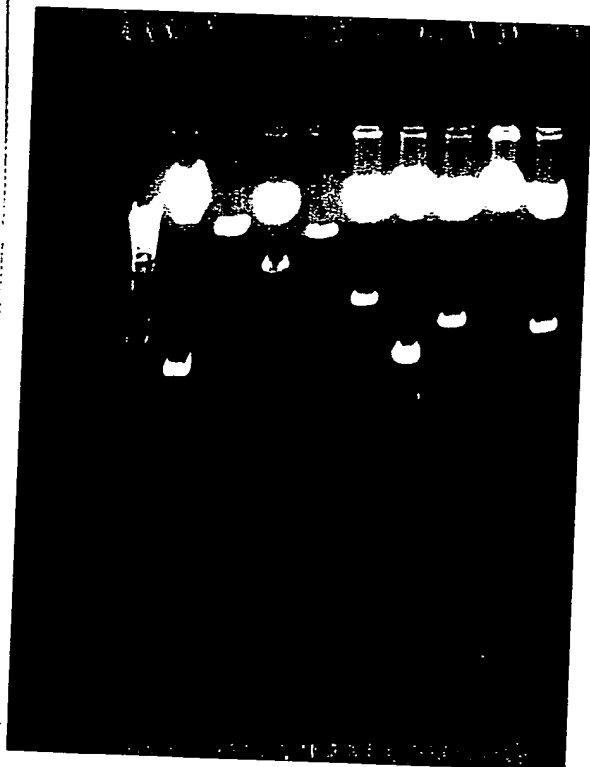


3-18-97

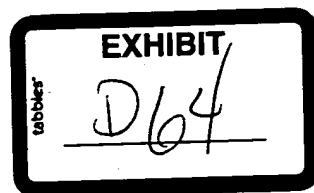
# Sequencing

- KD 9 ( p 21 , primer KD10 )
- KD 10 ( p 21 , primer KD11 )
- KD 11 ( p 111 , primer KD4 )
- KD 12 ( p 111 , primer KD5 )

Restriction pattern of Csl preps. of p101, p111.



- 1) 1 kb ladder
- 2) L2 / R1 + Nde1
- 3) p31 / R1 + Nde1
- 4) p101 / R1 + Nde1
- 5) p42 / R1 + Nde1
- 6) p111 / R1 + Nde1
- 7) L2 / Pac1 + Spe1
- 8) p101 / Pac1 + Spe1
- 9) L2 / Spe1 + Xba1
- 10) p111 / Spe1 + Xba1



3-21-97

Analysis of sequences KD 9, 10, 11, 12.

Everything is fine with p111. As predicted.

p21 - sequence with all primer is fine.  
- sequence with KD 10 primed shows that the 5' E. coli sequence is going after Ad nt 35858 and up to the end of sequence (~500 bp). → see next page

⇒ p626 and p82 are not responsible for virus  
Need to check p34611 for the same mutation.

EXHIBIT

D65

tabbles



3/27/97

3-20-97 Regues. (experiments of 02/05  
02/02)

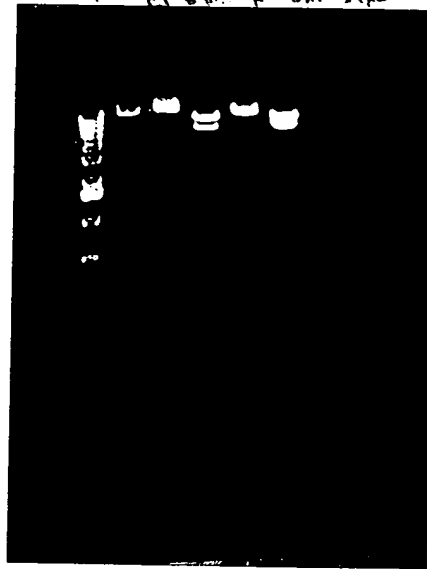
626M 1-12 (12)

626c 1-6 (6)

11 M 1-4 (4)

3-25-97

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



1) Colider 1KB

buffer-MC

2) 1101/1107 DNA / EcoRI + SpeI

3) p82

4) p82 / Bst 1107 I

5) p82 / XbaI

6) p82 / Bst 1107 I + XbaI

There is Bst 1107 I site in p82  
at AA 29012!

2nd is after SOB promoter!

3-27-97

Transfection 293 with p101, p111 + 1101/1107 / EcoRI + SpeI  
8X Gen dishes.

3 dishes 101 (for 1 dish) 250 µl NEBS 2X + 200 µl H<sub>2</sub>O + 10 µl 101

3 dishes 111 (for 1 dish) 250 µl NEBS 2X + 200 µl H<sub>2</sub>O + 10 µl 111

2 dishes control (for 1 dish) 250 µl NEBS 2X + 200 µl H<sub>2</sub>O

+ 10 µl 1101/1107 + 25 µl GCl<sub>2</sub>.

EXHIBIT

tabler

D66

4/7/97

Plaque assay of 544 on 253.

309 - ( $\sim 2$ ) - plaques are confluent (no many  
(cell stock) plaques  
in dish)

1101/1107 ( $\sim 2$ ) - Same as with 309  
(CsCl and amide ligate)

544 (titer  $\sim 2$ ) is about 1-2 orders of magnitude  
lower than in wt/309, but  
plaques appeared  $\sim 1$   
day later than in wt ( $\sim$  day 5) and are  
a bit smaller.

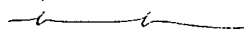
(day 16 size  $\sim 8$  mm)

7001 (CsCl) (titer is 1-2 orders  
 $\sim 2$  of m. lower than predicted for wt)  
plaques are tiny (day 16  $\sim 1-2$  mm diameter)

4/8/97

Ligation

12 ex 1) 82 (10  $\mu$ l) + PCR product ITR (10  $\mu$ l)  
Bst (part) + Xba from 140  
(49, 16, 17)

2) 87 (5  $\mu$ l) + 

4/16/97

Ligation 12 ex: vector 87/Xba + Bst (part) + CIP  
82/Xba + Bst + CIP  
upper fragment.  
82/Xba + Bst + CIP  
lower fragment

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

tabbies

D67

4/17/97 Sequencing

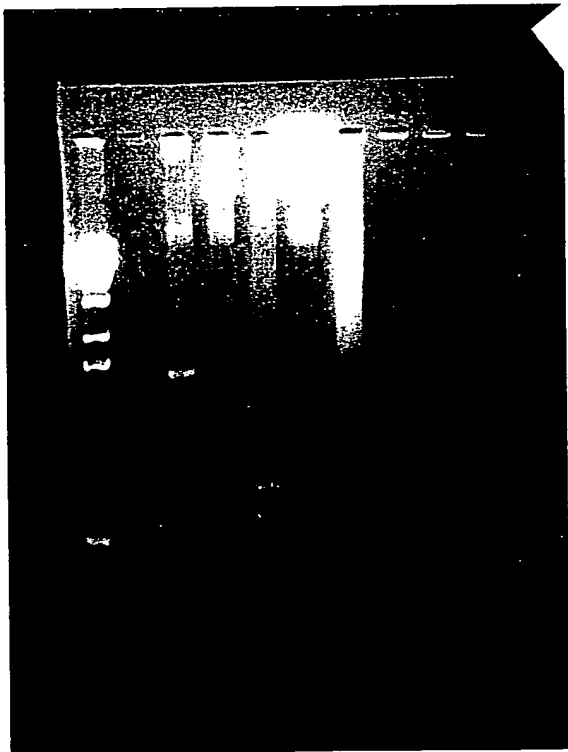
W) (14) - (pB4G11, primer KD10)

W) (15) - (pB4G10, primer KD10)

W) (16) - Mopman's Gd4 large plasmid, primer W) 16

W) (17) - p82, primer W) 10

W) (18) - p111, primer W) 10.



Link maps of 101-1  
and 111-1.

PCR, primers 1,5.

1) ladder.

2) blank

3) pB61wo.

4) p101

5) 101-1

6) p111

7) 111-1

p101, p111, <sup>(111-1)</sup> PCR product  
work.

for 101-1 product is

as expected for 111

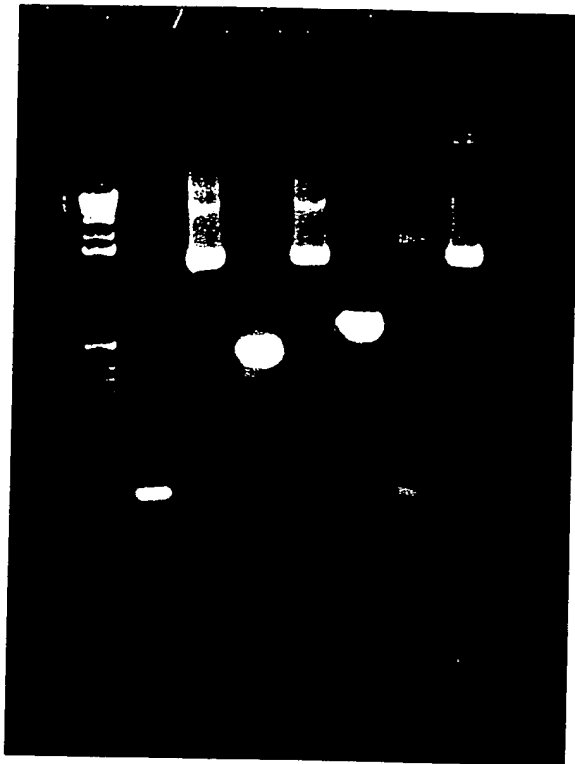
Mixed rules 101-1, 111-1  $\Rightarrow$  ? or what?

EXHIBIT

D68

tabbles

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



PCR, primers 1, 7.

- 1) Ladder
- 2) Blank
- 3) 140
- 4) 54
- 5) p101
- 6) p111
- 7) 101-1
- 8) 111-1

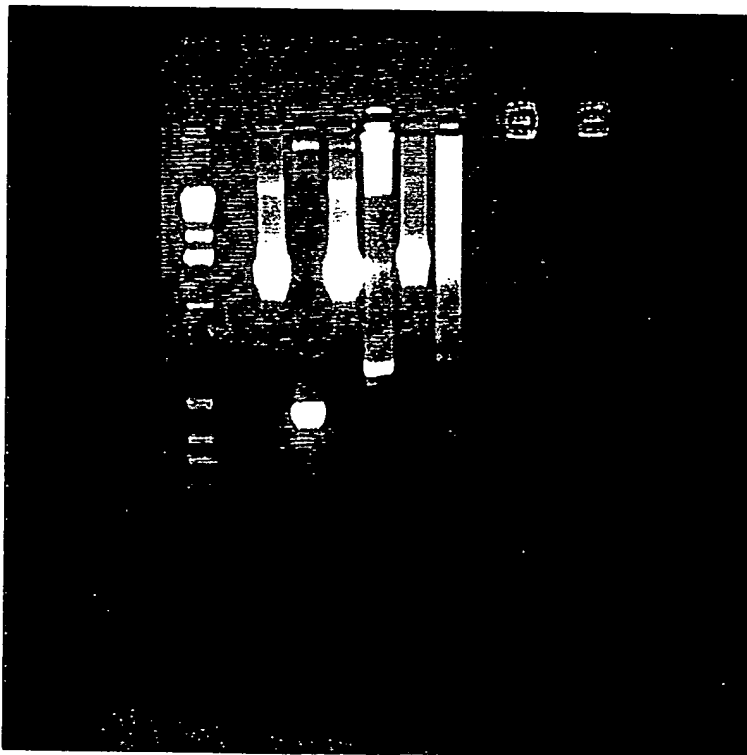
in 111-1 band is as low  
(or 101)

in 101-1 band is higher  
than wt.

4/21/97

PCR, primers 1, 7.

- 1) Ladder
- 2) Blank
- 3) p140
- 4) p54
- 5) p101
- 6) p111
- 7) 101-1
- 8) 111-1



Conclusion:  
this time everything  
is as expected.

- 9) 11-2
  - 10) 11-3
  - 11) 11-4
  - 12) 101-1
- } plagues

EXHIBIT

D69

1/22

## Sequencing

u) 19 - Chp's pED vector, primer LFC IIb

u) 20 - pB4G10, primer KD10.

u) 21 - p82, primer u) 16

u) 22 - p82, primer u) 17

u) 23 - p21, primer u) 16

u) 24 - p21, primer u) 17

## Results of previous sequencing

u) 14 (pB4G11, primer KD10) - sequence terminates on same point where the sequences start in p21.

u) 15 (pB4G10, primer u) 10) - same as ↑

u) 16 (Meyers large GAL4 plasmid) - same as ↑  
But there is some sequence which is what?

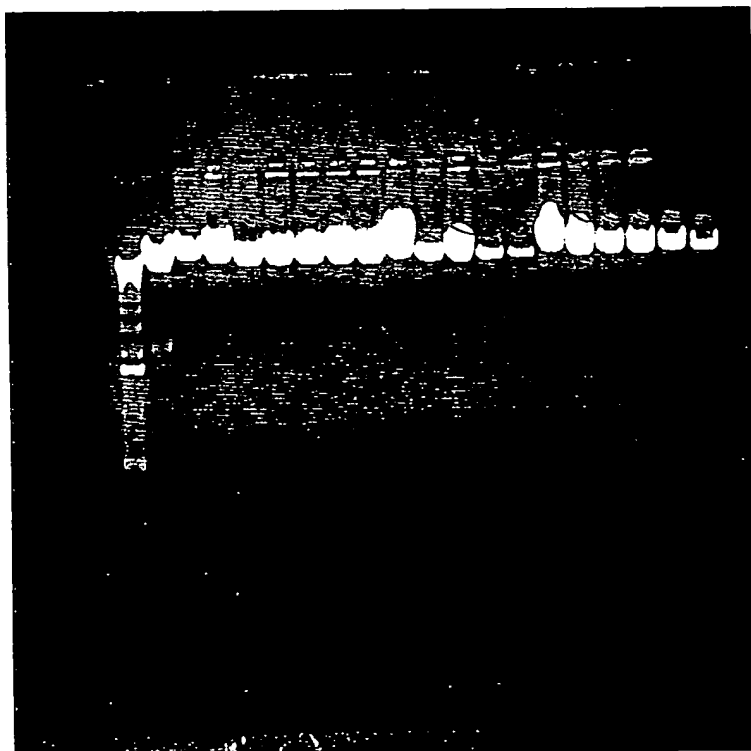
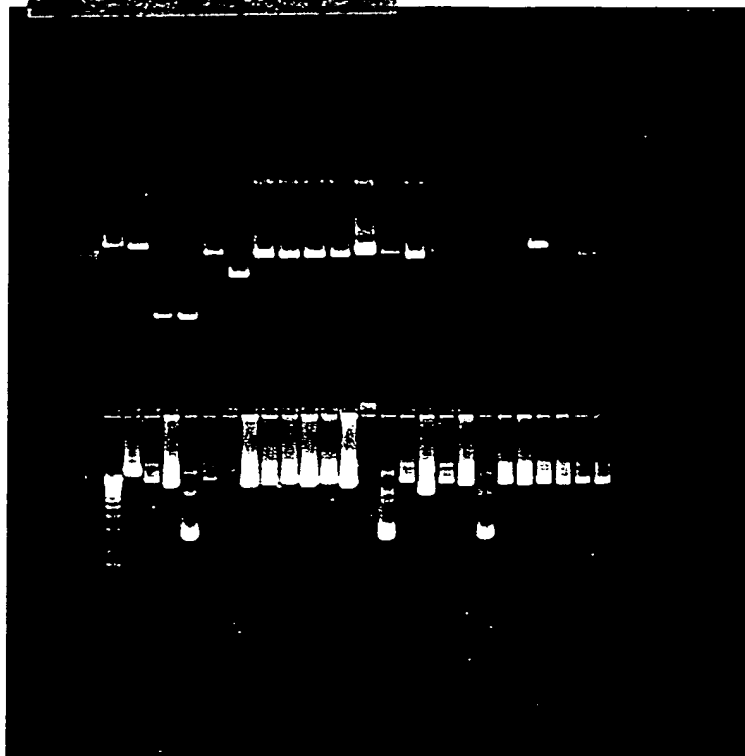
u) 17 (p82, primer u) 10) - sequence of 30-b promoter, need to repeat with u) 16 primer.

u) 18 (p111, primer u) 10) - sequence is perfect as predicted.

Sequences 19-24 - early termination everywhere  
⇒ new protocol doesn't work.

EXHIBIT

D70



4/22  
mini preps of 12 ex.

- 1) ladder,
- 2) p82
- 3) 87-1
- 4) 87-2
- 5) 87-3
- 6) 824 ex1 (1-9)
- 7) 824 ex2 (1-12)
- 8) ↓
- 9) ↓

- 1) ladder.
- 2) p82.
- 3) 82 L ex1 (1-12)
- 4) ↓ 82 L ex2 (1-12)

4/23 Restriction  
analysis of clones  
12 ex

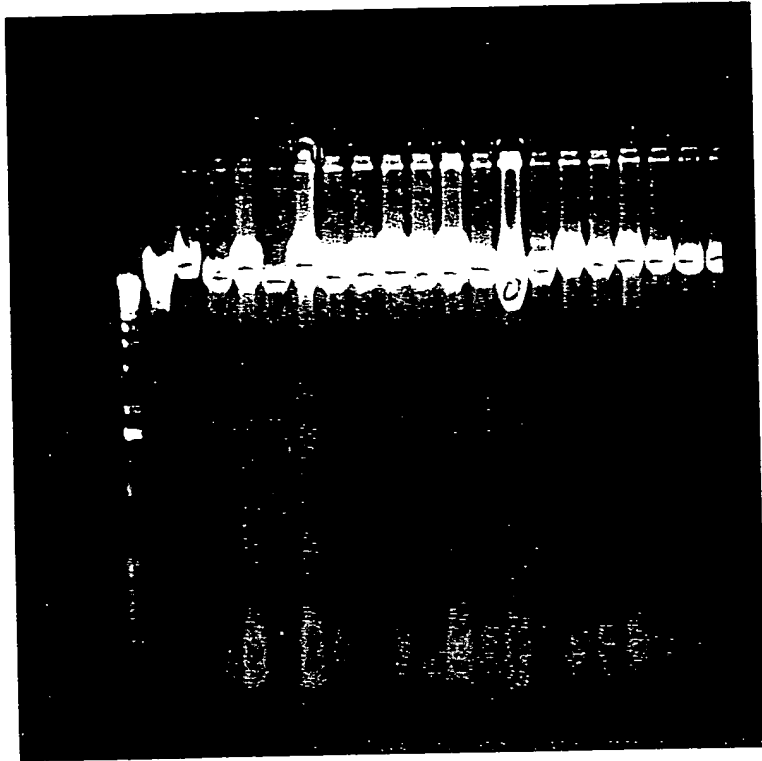
- 1) ladder
- 2) p82, R1
- 3) p82, X63
- 4) 87-1
- 5) 824-1
- 6) 824-3
- 7) 824-4
- 8) 824-5
- 9) 4-6
- 10) 4-7
- 11) 4-8
- 12) 4-9
- 13) 42-1
- 14) 42-4
- 15) 42-5
- 16) 42-7
- 17) 42-8
- 18) 42-9
- 19) 42-10
- 20) 42-12

Eco RI

Conclusions: 82/R1 -  
partial digestion.

clones - no small bands.

2 possibly recombinants,  
Marked bands have lower MW than p82/X63.



- 1) Ladder
- 2) p82/R1
- 3) p82/X63
- 4) L1-1
- 5) L1-2
- 6) L1-4
- 7) L1-6
- 8) L1-7
- 9) L1-8
- 10) L1-9
- 11) L1-10
- 12) L1-11
- 13) L2-2
- 14) L2-3
- 15) L2-4
- 16) L2-5
- 17) L2-7
- 18) L2-8
- 19) L2-9
- 20) L2-10
- 21) L2-11
- 22) L2-12

Conclusion: p82 - partial  
R1 digestion.  
clones - no small bands.

L2-5 - exclude from further analysis.

Try NdeI - XbaI ~~hydro~~ digestion.

expect 5 kb fragment in rec;  
6 kb fragment in 82 (wt)

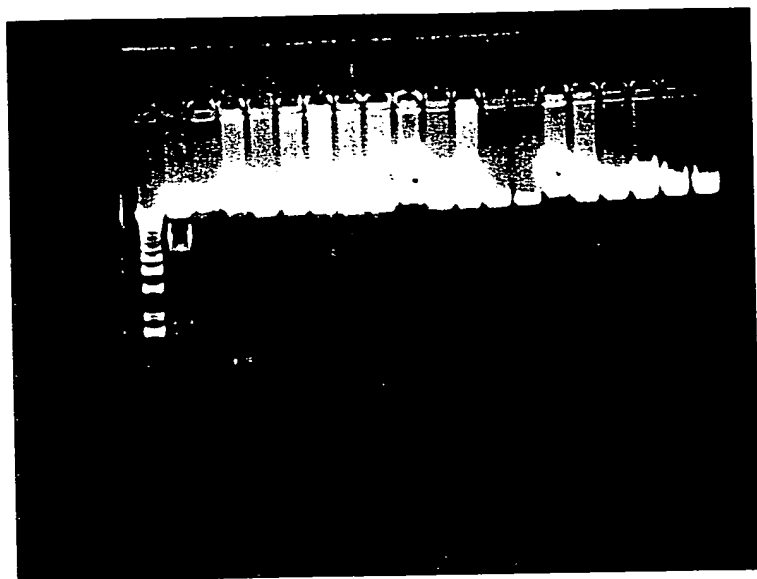
EXHIBIT

D72

4/29

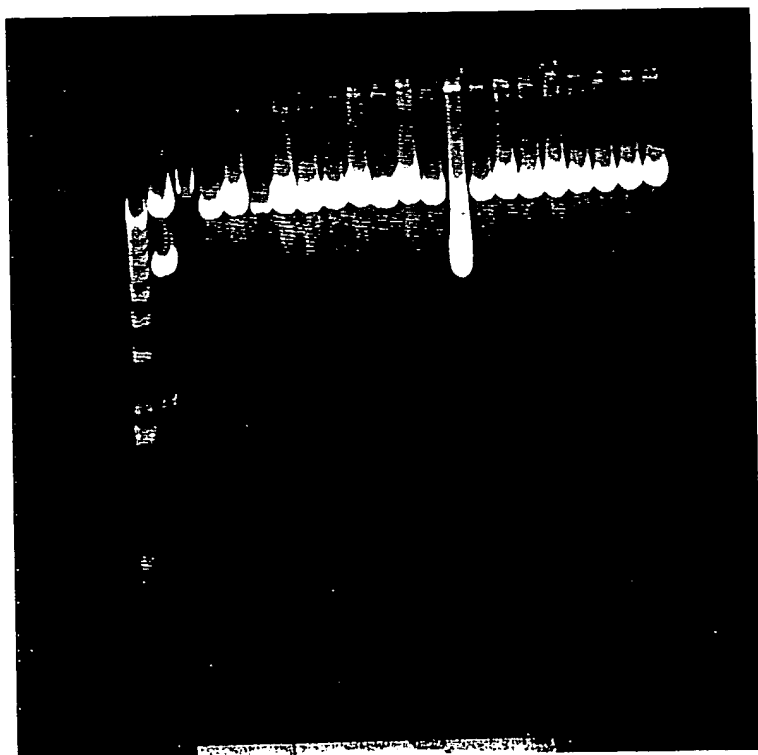
Same as previous 2  
pictures,

- 1) ladder
- 2) p82 / Xba + Nde
- 3) p82 / Nde
- 4) clones / Xba + Nde



Conclusion → all these  
clones are junk  
start cloning at 170  
into 223 where Bst<sup>III</sup>  
is unique, then put  
into resulting plasmid  
ben-Nde fragment from  
pRG140 (uniqueness  
again).

Probably, most of them  
are dt Bst - Bst clones?  
But where is vector  
background?



EXHIBIT

D73

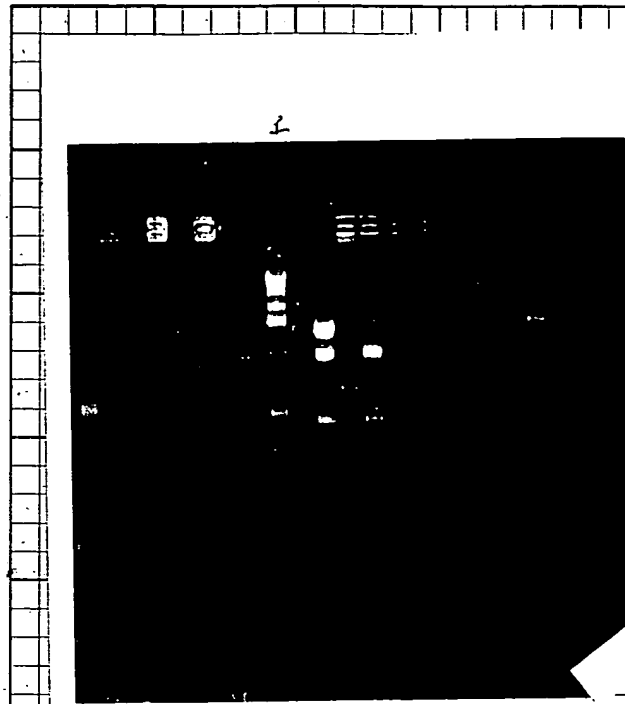
tabbies

22-141 50 SHEETS  
22-142 100 SHEETS





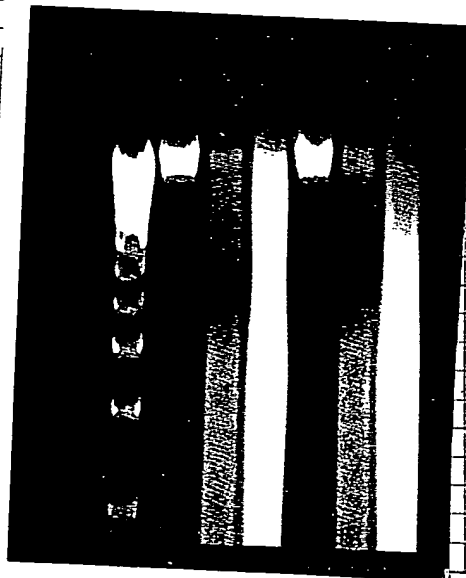
KDZ is made  
4/24/97



4/24

Restriction digestion  
of PCR products  
with Pst I  
(PCR primers 1, 7)

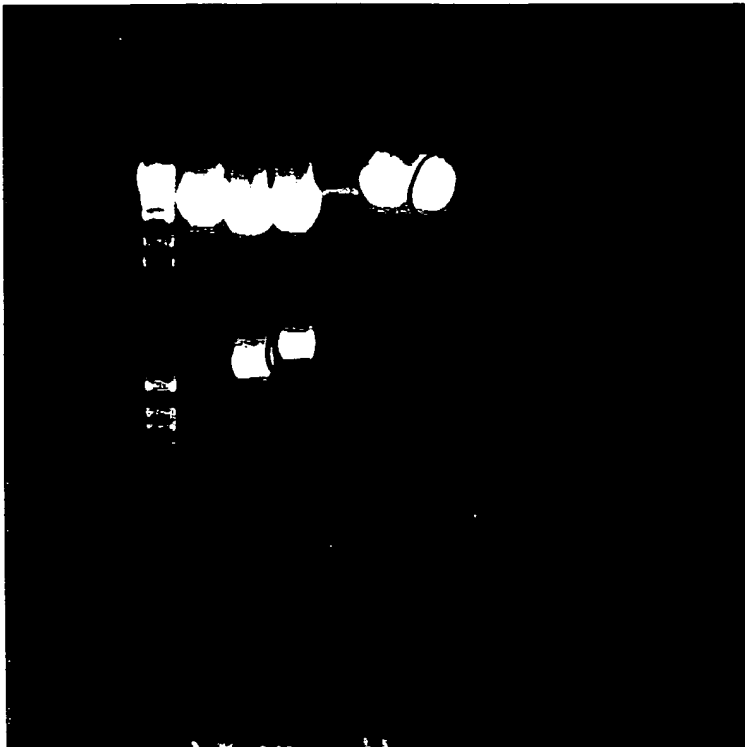
- 1) Ladder
- 2) p101
- 3) p101
- 4) p111
- 5) p101-1



- 1) Ladder
  - 2) p101/p101-1
  - 3) p101-1
  - 4) p111-1
  - 5) p101/p101-1
  - 6) p101-1
  - 7) p111-1
- Pst I  
Bam HI  
Xba I

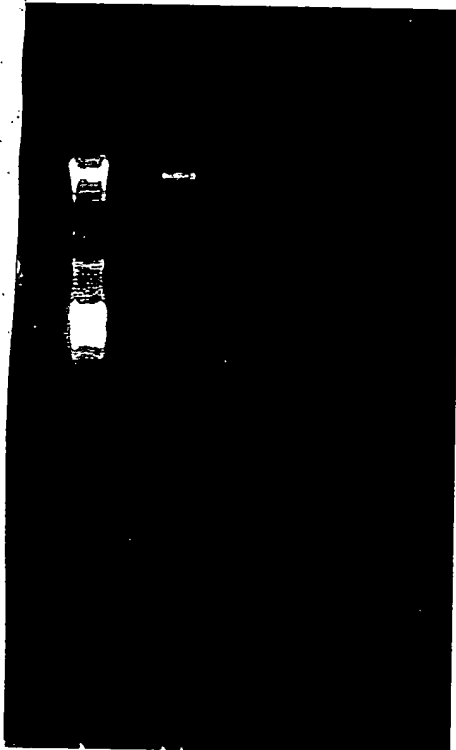
Conclusion: p101-1 is rec.  
p111-1 ?

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



4126.

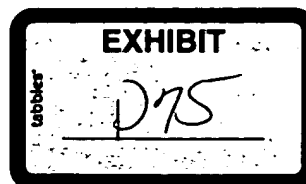
- 1) Ladder
- 2) pCR11/gp19uads7 (Ferry) / Kp1
- 3) Bam + Xba
- 4) pCR1/gp19u (Ferry) / EcoR1
- 5) pCDNAS / EcoR1
- 6) pCDNAS / Bam + Xba



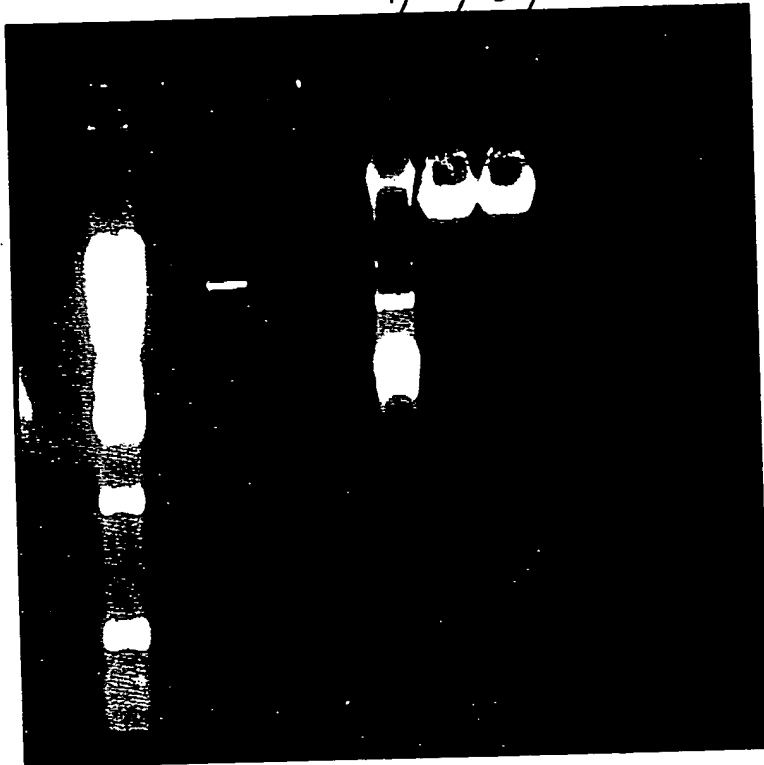
- 1) Ladder
- 2) Sp19uads Bam-Xba fr.
- 3) pCDNAS Bam-Xba vector.

Ligation:

- 1) vector 5 $\mu$ l
- 2) vector lig 5 $\mu$ l
- 3) vector + insert  
5 $\mu$ l 5 $\mu$ l



1) 2/31



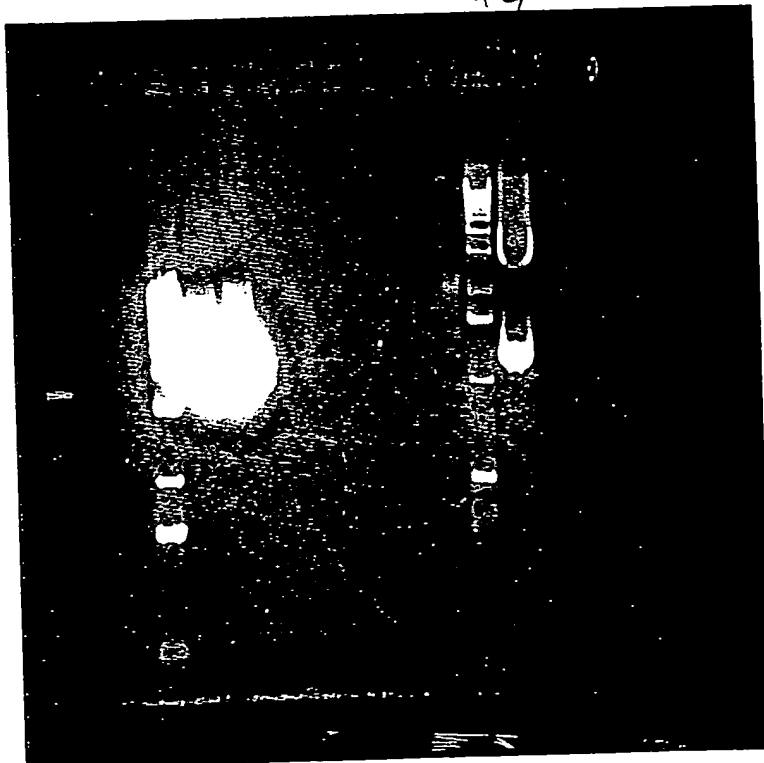
- 1) Colde
- 2) PCR 18p19u AdS/Ben + Bst11071
- 3) - - - - - /Ast11071 + X6e

It doesn't  
look like AdS 7p19u.

check with Pst1  
check with Terry!

4127

11124



- 1) Colde
- 2) PCR 18p19u AdS / Pst

there is a small  
fragment!

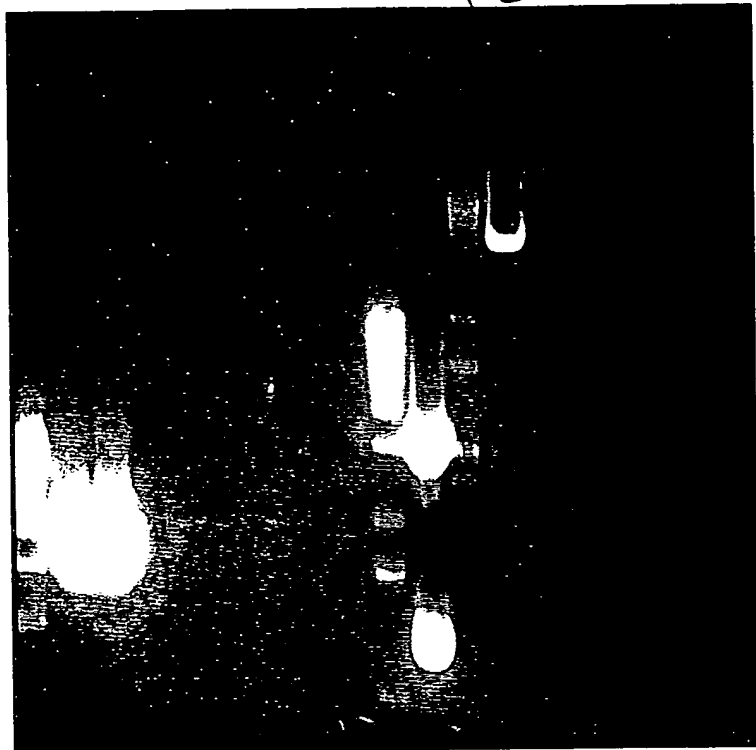
this is Ad2 gp is a  
in reverse orientation!?

check with Vind 141

EXHIBIT

D76

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



12

4/18.

1) ladder.

1) pcr4 / 8p4 / HindIII

~ 500 bp fragment?

4/18.

PCR / 8p19k

restriction digestion

1) ladder

2) pcr1 / 8p19k

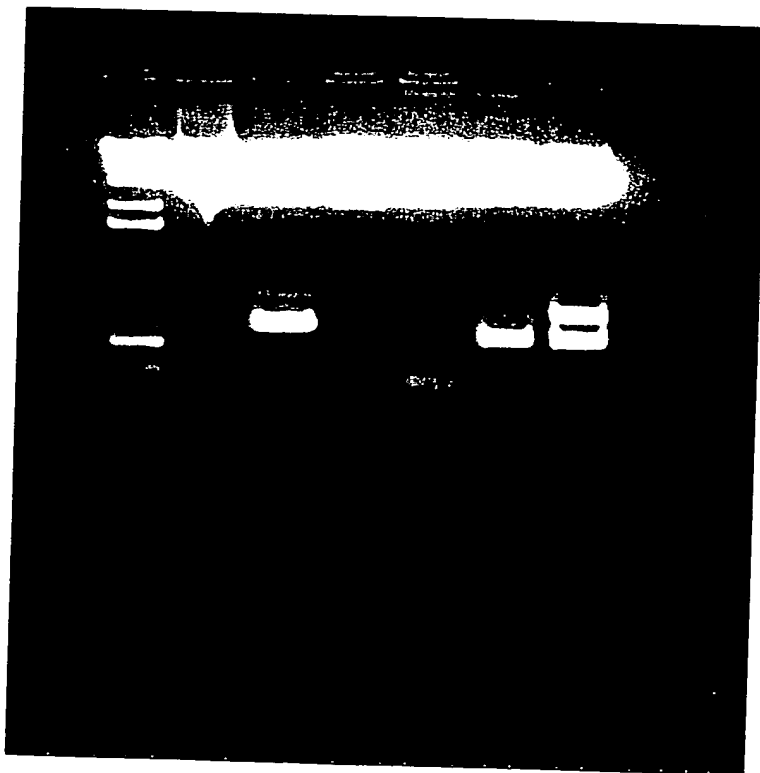
3) ——— / EcoRI

4) ——— / KpnI

5) ——— / PstI

6) ——— / HindIII

7) ——— / HindIII  
8p19k



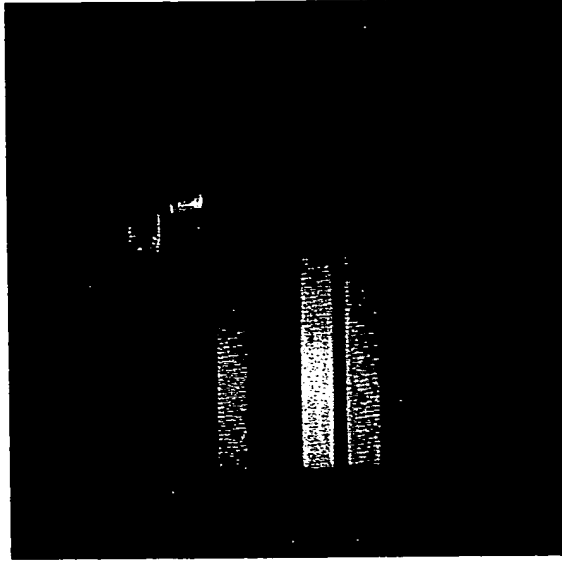
EXHIBIT

D77

tabbies

KO3 is made  
4/30/37

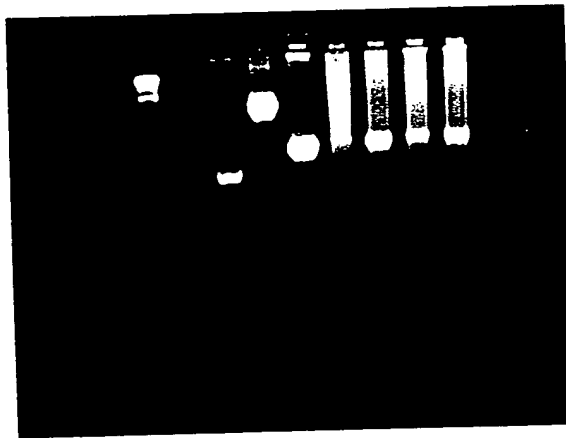
4/29/97 Restriction digestion of 611 plasmids



- 1) Ladder
- 2) 1101/1109 DNA
- 3) 111-1 (new) → Bam HI
- 4) 111-2 → Xba I
- 5) 111-3
- 6) 111-4

111-2, 3, 4 - look  
like recombinants

4/30/97  
PCR analysis of  
611 plasmids,  
primers K91, K97



- 1) Ladder
- 2) blank
- 3) p54
- 4) pEG1600
- 5) p111
- 6) 111-1
- 7) 111-2
- 8) 111-3
- 9) 111-4

Conclusion is → all plasmids are

EXHIBIT

D78

tabbies

4/30. Ligation:

cloning experiment 14. (14 ex).

1) Vector p223 / Bst 11071 + Xba I (PEB prep.)

2) Vector p223 / Bst 11071 + Xba I (Wikit prep.)

3) Insert 1101/1107 PCR ITR / Bst 11071 + Xba I  
(without gel purification).

S/S. Comparing growth properties of: (plaqueing efficiency)

54 (4)	Controls	309
101 (-1)		1101/1107
111 (-1)		7001
111 (+2)		m1 m41 (pm 734.1)

8/6/97

Minipreps of 14 ex.

1) ladder

2) p82

3) p223

4) 12ex-1

5)

6) 14ex 1 (1-3)

7)

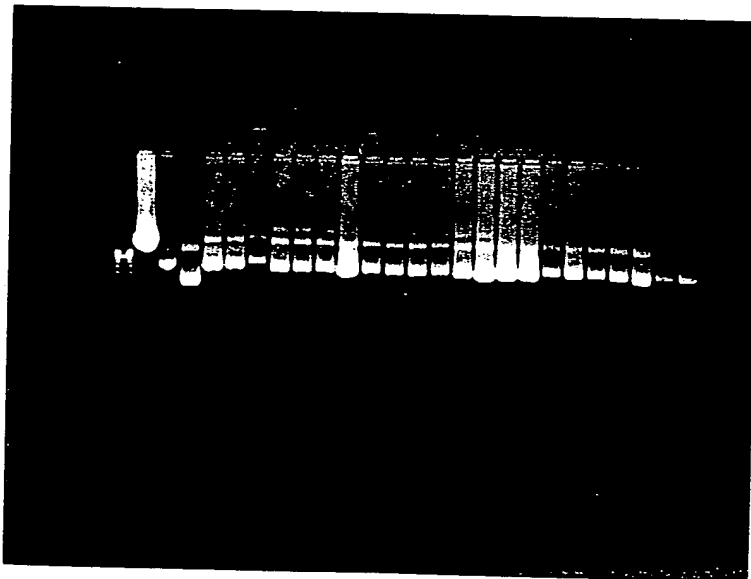
9) 14ex 2 (1-20)

10)

↓

26)

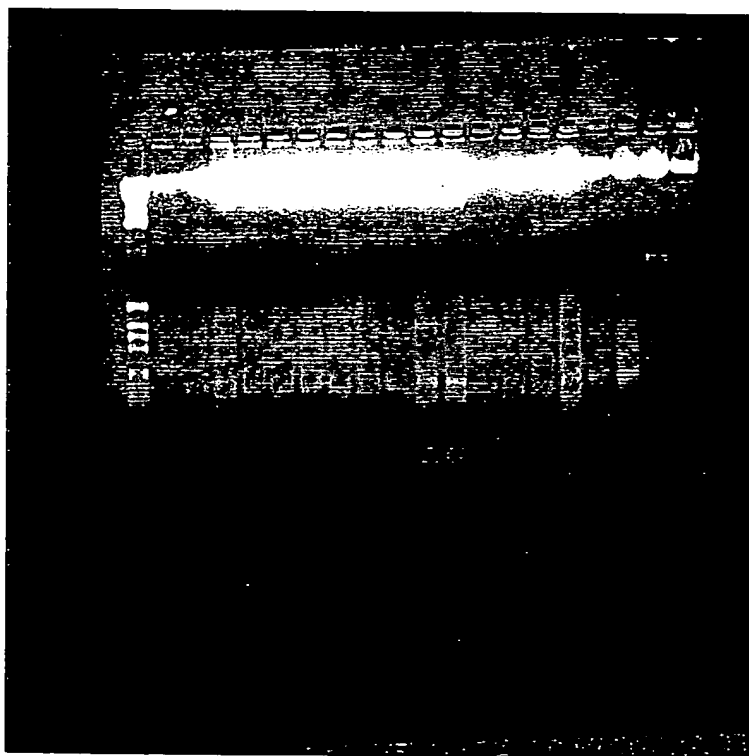
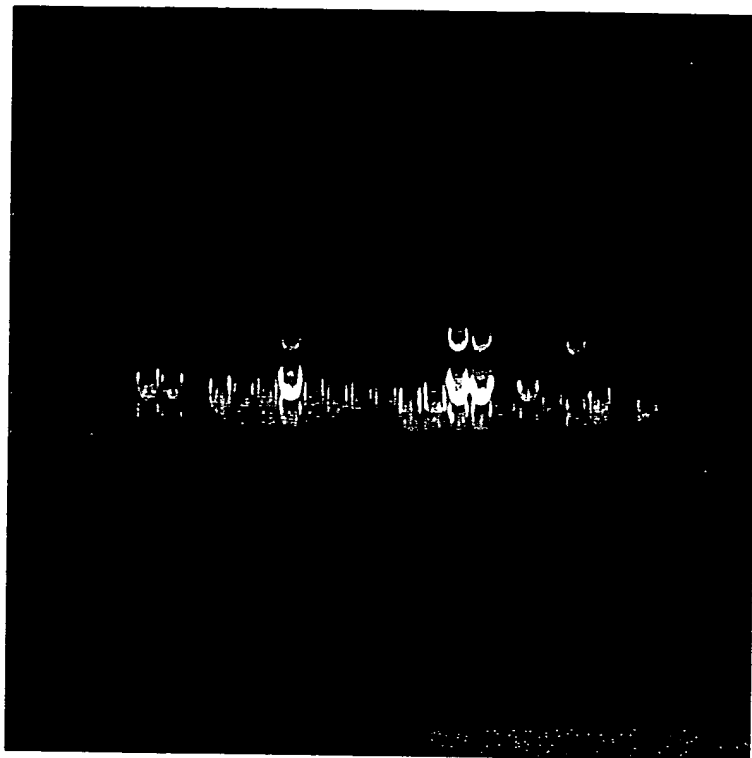
⇒ 12ex-1 - junk.



EXHIBIT

D79

tabler



4/6/97

1) Calder

Xba + Nde

restriction of  
~~2~~ 14 ex clones

2) p223

141 (A3)

142 (1-20)

Xba +  
Nde

Conclusion:

141-3

142 -

- 1, 2, 3,

5, 6, 7, 8, 9, 10,

13,

15, 16, 17, 18, 19

on mutants

4/7/97

1) Calder

2) p223

3) 141 (3)

4) 142 (1)

5) 142 (8)

6) 142 (5)

7) 142 (5)

8) 142 (6)

9) 142 (7)

10) 142 (8)

11) 142 (9)

12) 142 (10)

13) 142 (13)

14) 142 (15)

15) 142 (16)

16) 142 (17)

17) 142 (18)

18) 142 (19)

19) 141 (11) (wt)

All rec  
clones  
from A  
are  
rec-ts.

bs 217T  
(bs 1107T)  
+ Xba L

20/142/19  
wt)

4/8

# Sequencing

u) 25 - p141(3) - primer u) 16  
 u) 26 - p141(3) - u) 17  
 u) 27 - p142(1) - u) 16  
 u) 28 - p142(1) - u) 17

sequencing  
Didn't work

sequence is OK.

4/14. properly vectors pBSSK(+)/Sma I McI 30°C  
p142(1) Bam + Nde. Mc

rec 700, pm 734.1 predicted  
fragments length for Srf + Nde  
digestion:

19549  
 7981  
 4846  
 3559

## Ligation:

Cloning exp 15 (p142(1) Bam + Nde + pEG160  
Bam - Nde Bfr)

Cloning exp 16 (pBSSK Sma + rec 700 / Srf + Nde + T4 pol)

Cloning exp 17 (pBSSK Sma + pm 734.1 / Srf + Nde + T4 pol)

5/16. Results of transform. DMS with 15, 16, 17 ex:

15 v - 1  
 15 vL - 14  
 15 ex - 25

15-2 - MW as 82  
 others either wt 142-1 or small  
 plasmids

16 v - 0  
 16 vL - 2R + 1W  
 16 ex - 5R + 19W  
 17 ex - 7W

all wt or same size

EXHIBIT

D81

tabbies





12 3 4



4/20

- 1) p82
- 2) p152

father m...

1) ladder

- 2) p82 } Bonnell
- 3) p152 } Ndr1
- 4) p82 } Ndr1
- 5) p152 } X631

Conclusion: 152 is ok,  
making cell prep.

5/25 Mappings of 17ex

17ex - 2 (Kleiman  
- exercise fragment from jet)

1) ladder

- 2) p82 (r)
- 3) 17ex

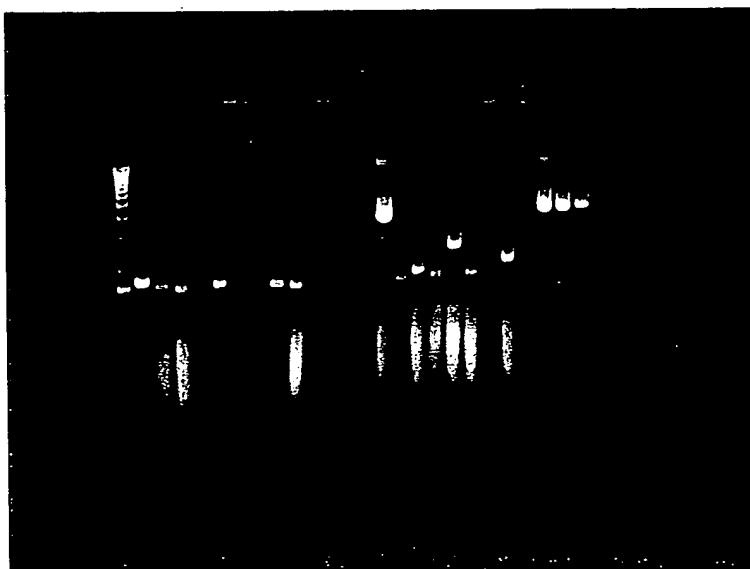
↓  
1-12

17ex2

1-12

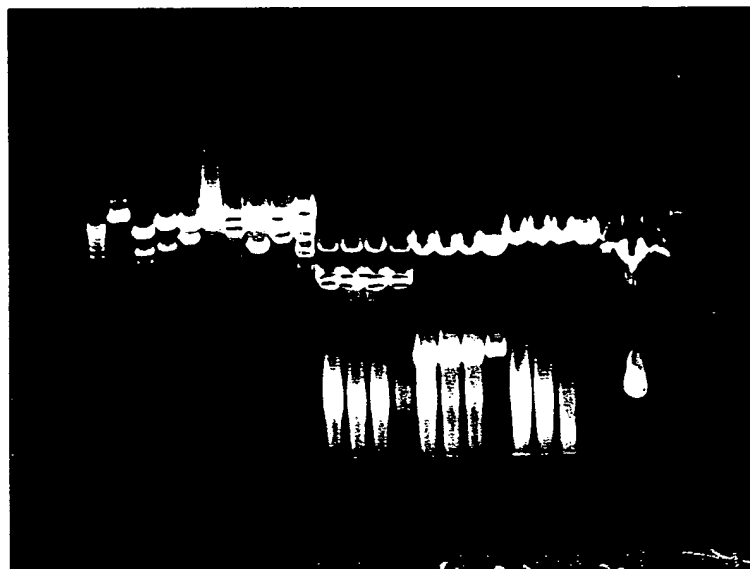
Take 172-1, 10, 11, 12

172-5 } possibly  
172-8 } some J.K.



EXHIBIT

D82



5/29/97

- 1) ladder
- 2) p82
- 3) p82 / Bam + Nde
- 4) p82 / Nde + Xba
- 5) p82 / Bst 1107 I
- 6) p152
- 7) p152 / Bam + Nde
- 8) p152 / Nde + Xba
- 9) p152 / Bst 1107 I
- 10) 734.1 / Srf + Nde
- 11) 1721
- 12) 17210
- 13) 17211
- 14) 17212
- 15) 1721
- 16) 17210
- 17) 17211
- 18) 17212
- 19) 1721
- 20) 17210
- 21) 17211
- 22) 17212
- 23) pC19p191C / Xba + Xho
- 24) 1101/1107 / Spe + EcoRI

Conclusion: 152 is as expected.

17ex clones have correct insert in some (direct) orientation (EcoRI digestion) ~~Xba~~ do not cut insert due to methylation of Xba site.

The insert could be cut out with BamI-SbfI to clone it into pCDNA3.1 BamI-XhoI sites.

EXHIBIT

D83

5/28/97

Transfection 1101/1107 EcoRI + SpeI + p152

6 dishes 293/TTC

8 dishes 293

293 TTC

2 dishes - control (1 ml)

2 - TTC (1 ml)

4 dishes - experiment 2 - + Cuv/TTC (1 ml)

5/29/97

Cloning 18 ex:

172 BamI + SalI + XbaI (last)  $\Rightarrow$  3.5 kb (A)  
fragment (1.1 kb) + CIP  $\Rightarrow$  (buffer D)

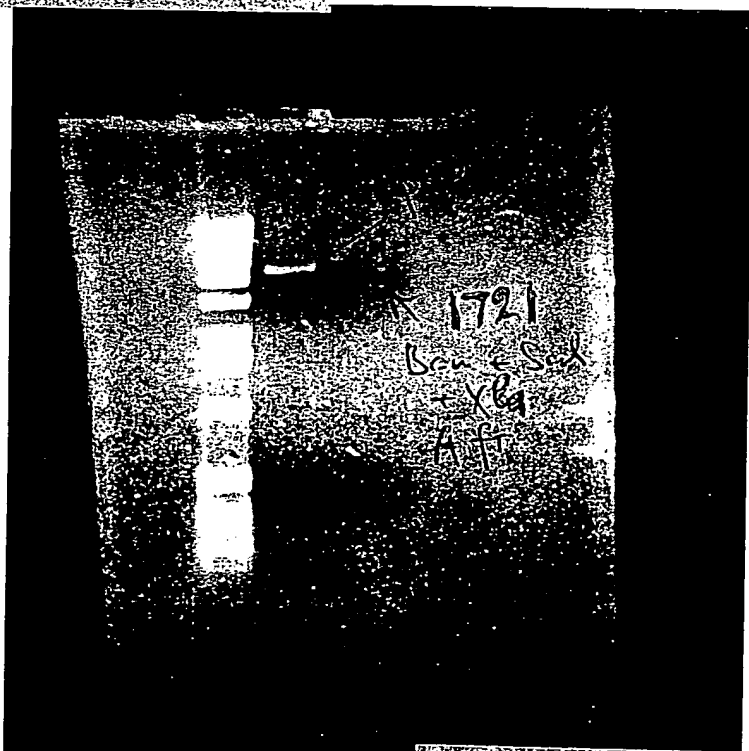
pCDNA3.1 BamHI XhoI  $\Rightarrow$  vector

700

50 kb

$\downarrow$  (buffer D)

V(SM) - 0, VL  $\approx$  30; ex  $\sim$  300; LL (10  $\mu$ l) - 0



EXHIBIT

D84

First cell culture  
experiment with  
KO1, 2, 3  
5/15/97

5/15/97 Plaque assay of E3 viruses 293 cells

Day	Number of plaques	plaque size (mm)										
Virus	Approximate		3	4	5	6	7	8	10	19		
309	(~2)	1/1	8/2	~100/1-5	~300/1-5	~500/1-5	same	same	complete CPE	~1000	complete CPE	
	(~1)	0/0	0/0	~20/1-2	~50/1-5	~100/1-5	same	~100	~100	~200		
110/1107	(~2)	0/0	0/0	~50/1-2	~30/1-5	~50/1-5	same	~100	~100	~500		
	(~1)	0/0	0/0	5/1	14/1-2	10/1-2	same	~30	~30	50		
7001	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0		
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0		
pm T34.1	(~4)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	~30		
	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0		
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0		
544	(~4)	0/0	0/0	10/1	18/1-2	20/1-2	30/1-2	~60/1-2	~60/1-2	~100		
	(~3)	0/0	0/0	0/0	1/1	4/1-2	same	9/1-2	9/1-2	30		
101-1	(~4)	6/1	15/1-2	>100/1-5	>300/1-5	>1000	same	complete CPE	complete CPE	complete CPE		
	(~3)	0/0	0/0	~50/1-5	~80/1-5	>100	same	>300	>300	>300		
	(~2)	0/0	0/0	~15/1-5	~30/1-5	>30	same	>70	>70	100		
111-1	(~4)	6/2	9/2-8	>100/1-10	>300/1-10	>1000	same	complete CPE	complete CPE	complete CPE		
	(~3)	0/0	1/8	~50/1-10	~80/1-10	~80	same	~300	~300	~300		
	(~2)	0/0	0/0	1/1	30/1-3	~50	same	~150	~150	~250		
111-2	(~4)	0/0	8/8-5	20/1-2	50/1-5	50	same	~100	~100	>100		
	(~3)	0/0	1/1	10/1	20/1-5	30	same	~50	~50	>50		
	(~2)	0/0	0/0	0/0	7/1	12	same	23	23	28		
309	(~2)	CCPE	approximate									
	(~1)	CCPE										
110/1107	(~2)	CCPE										
	(~1)	CCPE										
7001	(~3)	2	3	3	3	3						
	(~2)	0	1	3	3	5						
pm T34.1	(~4)	~50	~60	~60	~70	~70						
	(~3)	4	7	9	10	16	23	30				
	(~2)	0	0	1	1	2						
544	(~4)	~100	~100	~100	~100							
	(~3)	33	33	33	33							
101-1	(~4)	CCPE										
	(~3)	CCPE										
	(~2)	CCPE										
111-1	(~4)	CCPE										
	(~3)	CCPE										
	(~2)	~50	50									
111-2	(~4)	>600	~100									
	(~3)	750	~50									

EXHIBIT

D85

5/15 Rec ADP in ES plaque development on AS44.

deg = 5 6 7 8 <sup>Friday</sup> <sup>Saturday</sup> <sup>Final</sup>

12

(12+1)

(14)

(17)

(18)

309 (~1) 12 52 ~100 ~100 ~100 CCPE

(~0.1+1) 1 5 14 19 20

(~0.1-2) 3 7 19 26 27

104/107 (~1) 0 0 3<sub>small</sub> 8 15

(~0.1+1) 0 0 1 3 3 5 6

(~0.1-2) 0 0 0 0 1 2 2

7001 (~3) 0 0 0 0 0 0 2 2

(~2) 0 0 0 0 0 0 0

734.1 (~3) 0 0 0 0 0 8 8 11 15

(~2) 0 0 0 0 0 4 5 6 9 10

101-1 (~3) 1 4 48 60 ~60

101-1 (~2) 3? 7 20 30 34

101-1 (~1) 0 2 3 4 7 9

11-1 (~3) 0 6 35 50 ~50

(~2) 0 0 5 9 16

(~1) 0 0 2 4 4

11-2 (~4) 3? 30 70 100 ~100

(~3) 2? 4 16 20 30

(~2) 0 0 1 4 4 6

(~1) 0 0 0 0 0 0

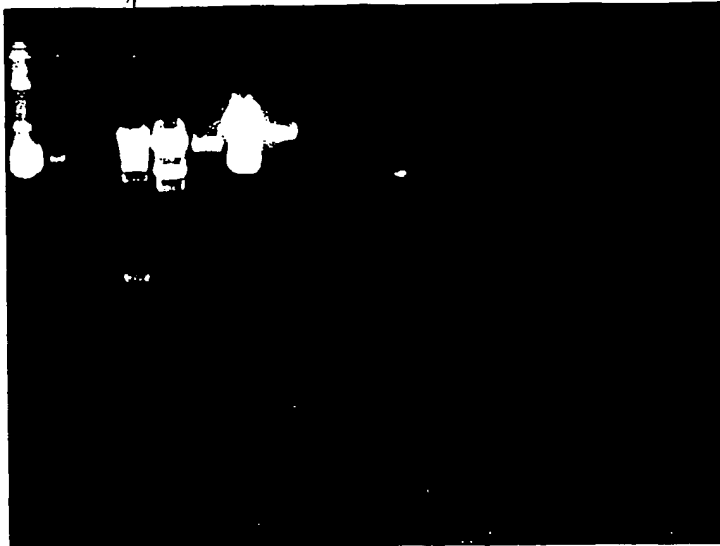


22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

D86

12 34



4120

- 1) p82
- 2) p152

rather than

7 ladder

- 2) p82 7 Bonnell
- 3) p152 7 Ndr

- 4) p82 7 Ndr

- 5) p152 7 X63

Conclusion: 152 is ok, making cell prep.

5/25 Mungreps of 17ex

17ex - 2 (Klempner)  
- excise fragment from gel

- 1) ladder
- 2) p82 (+)
- 3) 17ex

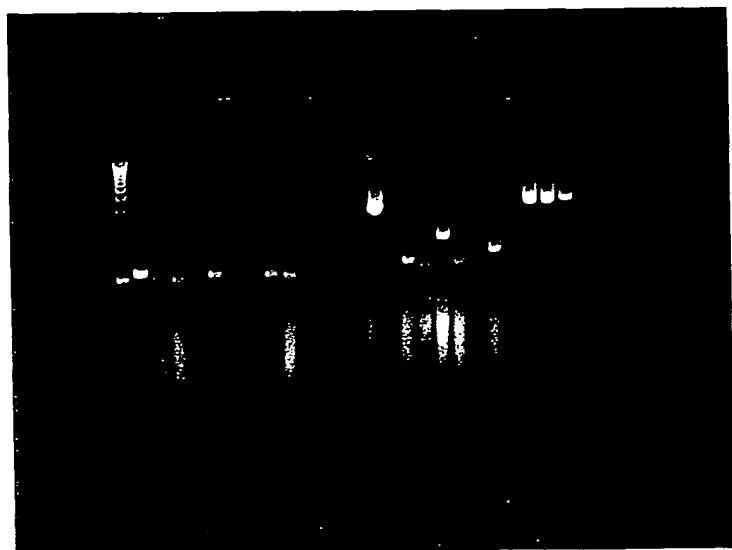
↓  
1-12

17ex?

1-12

Take 172-1, 10, 11, 12

172-5? } possibly  
172-8? } same junk.



EXHIBIT

D87

8544, 5/5/97, 5/27/97

Ray (Plagues)

virus: 5 6 7 8 9 10 11 12 13 14 15 16 17 18

302

4 12 33 45 47  
8.5 25 10 15 100

#

1101/1107

18 24 18  
19 159 185

207 21  
95 100

7001

734.1

12 13 11 24  
50 54 70 100

544

14 25 91 42  
133 53 147 100

101-1

3 8 23 34 41 43  
17 20 53 79 95 100

111-1

2 4 16 20 30  
17 113 53 66 100

EXHIBIT

D88

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



5/5/97 Player development on 295. (1. players)

Vins <sup>day</sup> 4 5 6 7 8 10 14 15 16 18 19 22 23 24  
305 10 25 50 100 100

1101/1107 8.3 16 50 83 100

7001

~~25 50 75 100~~ ?

734.1

~~(135 235 30 33 55 76 100)~~ !

544. 3 12 27 90 100

101-1. ① 15 30 70 100

111-1 ~~20 60 100~~ ?

111-2 21 36 69 84 96 100

5/15/97 ↑ Same, 1549.

5 6 7 8 9 12 13 14  
309 11 25 70 96 100

1101/1107 16 50 83 100

~~700~~  
734.1

~~70 76 100~~ ?

101-1 18 27 36 63 82 100

111-1 25 50 100

111-2 16 66 100

EXHIBIT

D89



5/15/97 Plaque assay of E.S. viruses 293 cells

Day - Number of plaques / plaques size (mm)		Approximate										
Virus (name) / index of magnitude		3	4	5	6	7	8	9	10			
309	(~2)	1/1	8/2	~100/1-5	~300/1-5	~500/1-5	Same	complete CPE	~1000			
	(~1)	0/0	0/0	~20/1-2	~50/1-3	~100/1-5	Same	~100	~200			
110/1107	(~2)	0/0	0/0	~50/1-2	~50/1-5	~50/1-5	Same	~100	~500			
	(~1)	0/0	0/0	5/1	10/1-2	10/1-2	Same	~30	50			
7001	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	2			
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0			
pm T34.1	(~4)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	~30			
	(~3)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0			
	(~2)	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0			
544	(~4)	0/0	0/0	10/1	18/1-2	20/1-2	30/1-2	~50 (tiny)	~100			
	(~3)	0/0	0/0	0/0	1/1	4/1-2	Same	8/1-2	30			
101-1	(~4)	6/1	15/1-2	>100/1-5	>300/1-5	>1000	Same	complete CPE	Complete			
	(~3)	0/0	0/0	~50/1-5	~80/1-5	>100	Same	>300	>300			
	(~2)	0/0	0/1	~15/1-5	~30/1-5	>30	Same	>70 Range	100			
111-1	(~4)	6/2	9/2-8	>100/1-10	>300/1-10	>1000	Same	complete CPE	complete CPE			
	(~3)	0/0	1/8	~50/1-10	~80/1-10	~80	Same	~300	>300			
	(~2)	0/0	0/0	10/1 (most)	30/1-3	~50	Same	~50 Range	>50			
111-2	(~4)	0/0	8/8-5	20/1-2	50/1-5	50	Same	~100	>100			
	(~3)	0/0	1/1	10/1	20/1-5	30	Same	~50	>50			
	(~2)	0/0	0/0	0/0	7/1	12	Same	23	28			
Size measurements		(15)	(16)	(18)	(19)	(20)	(23)	(24)	(25)			
309	(~2) CCPE	approximate										
	(~1) CCPE											
110/1107	(~2) CCPE											
	(~1) CCPE	30	60									
7001	(~3)	2	3	3	3	3						
	(~2)	0	1	3	3	5						
pm T34.1	(~4)	~50	~60	~60	~70	~70						
	(~3)	4	7	9	10	16	23	30				
	(~2)	0	0	1	1	2						
544	(~4)	~100	~100	~100	~100							
	(~3)	33	33	33	33							
101-1	(~4) CCPE											
	(~3) CCPE											
	(~2) CCPE											
111-1	(~4) CCPE											
	(~3) CCPE											
	(~2) SP	50	50									
111-2	(~4) >100	~100										
	(~3) >50	~50										
	(~2) 32	33										

First cell culture experiment with K01-2-3 5/15/97

EXHIBIT D90

First cell culture experiment with 101-2, 3  
5/15/97

EXHIBIT

D90

5/15 Fee ADP: 2 ES plaque development on ASU4

day = 5 6 7 8 Friday Saturday Feb.

(12+1)

(14)

(15)

(16)

CCPE

309 (~1)	12	52	~100	~100	~100	CCPE			
(~0.1+1)	1	5	14	19	20				
(~0.1-2)	3	7	19	26	27				
(~1)	0	0	3 small	8	15				
(~0.1+1)	0	0	1	3	3	5	6		
(~0.1-2)	0	0	0	0	1	2	2		
7001 (~3)	0	0	0	0	0	0	0	2	2
(~2)	0	0	0	0	0	0	0		
734.1 (~3)	0	0	0	0	0	8	8	11	15
(~2)	0	0	0	0	0	4	5	6	9
101-1 (~3)	1	11	48	60	~60				
(~2)	3?	7	20	30	34				
(~1)	0	2	3	4	7	9			
11-1 (~3)	0	6	35	50	~50				
(~2)	0	0	5	9	16				
(~1)	0	0	2	4	4			8	11
11-2 (~4)	3?	30	70	100	~100				
(~3)	2?	4	16	20	30				
(~2)	0	0	1	4	4				7
(~1)	0	0	0	0	0				



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

D91

5/21/97 Plaque assay of E3 viruses on ASUG;  
 (envelope) is dying, data are not quantitative.

Day,  
 plaques

6 7 8 12 14

300 (~2) ~30  
 (~1) 3

12 33

1107 (~3) 0  
 (~2) 0  
 (~1) 0

3 17  
 3 17  
 0 6

27  
 30  
 7

00 (~4) 0  
 (~3) 0

0 0  
 0 0

0 0  
 0 0

734 (~4) 0  
 (~3) 0

0 0  
 0 0

2 3

549 (~4) 14  
 (~3) 0  
 (~2) 0

24 239  
 0 2  
 0 0

3 3  
 0 0

101 (~3) 0  
 (~2) 0

11 28  
 3 11

12

111 (~3) 0  
 (~2) 0

0 3  
 0 7

5 14

111-2 (~5) 0  
 (~2) 0  
 (~1) 0

7 27  
 0 1

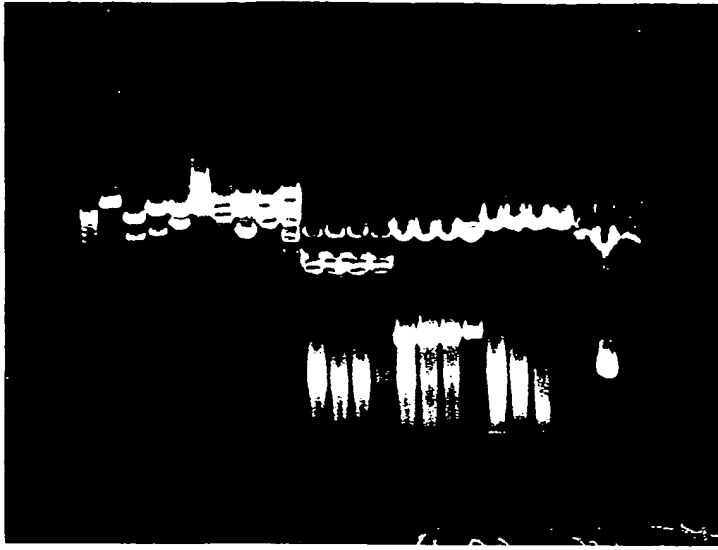
2 0

22-141 50 SHEETS  
 22-142 100 SHEETS  
 22-143 200 SHEETS  
 AMPAD

EXHIBIT  
 DG2

5/29/97

22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

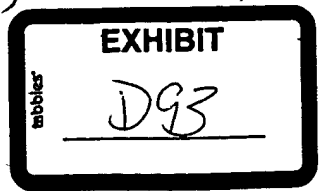


- 1) Edder
- 2) p82
- 3) p82 / Bam + Nde
- 4) p82 / Nde + Xba
- 5) p82 / bst 1107
- 6) p152
- 7) p152 / Bam + Nde
- 8) p152 / Nde + Xba
- 9) p152 / bst 1107
- 10) 734.1 / Srf + Nde
- 11) 1721
- 12) 17210
- 13) 17211
- 14) 17212
- 15) 1721
- 16) 17210
- 17) 17211
- 18) 17212
- 19) 1721
- 20) 17210
- 21) 17211
- 22) 17212
- 23) pC19p191C / Xba + Xho
- 24) 1101/1107 / Spe + EcoRI

Conclusion: 152 is as expected.

17 ex clones have correct insert in some (direct) orientation (EcoRI digestion) Xba doesn't cut insert due to methylation of Xba site.

The insert could be cut out with BamI-SbfI to clone it into pCDNA3.1 BamI-XhoI sites.



5/28/97

Transfection 1101/1107 EcoRI + SpeI + p152.

6 dishes 293/TTF

8 dishes 293

293 TTF

2 dishes - control (1 ml)

2 - TTF (1 ml)

4 dishes - experiment 2 - + Cuv/TTF (1 ml)

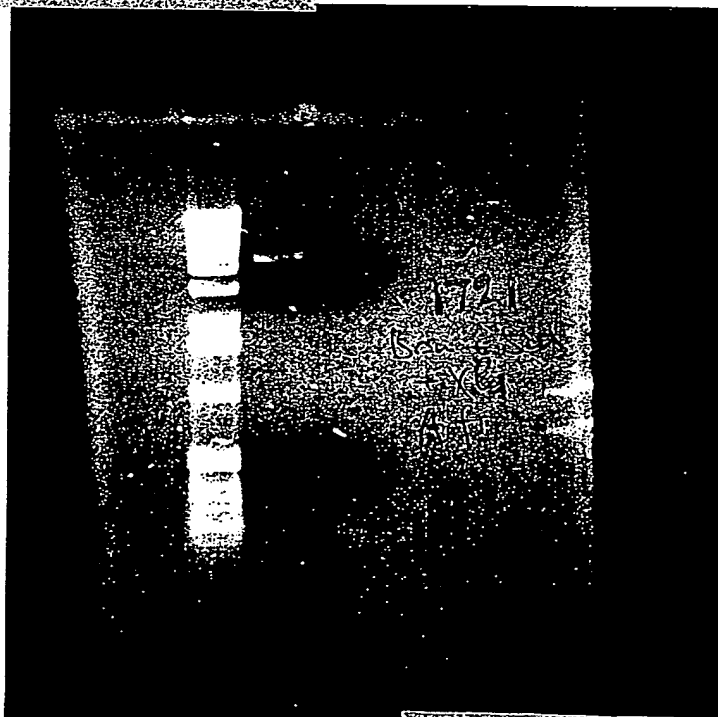
5/29/97

Cloning 18 ex:

172 BamI + SalI + XbaI (last)  $\Rightarrow$  3.5 kb (A)  
fragment (1 cut) + CIP  $\Rightarrow$  (buffer)

pCDNA3.1 BamI + XhoI  $\Rightarrow$  vector  
720 5.0 kb  $\Rightarrow$  (buffer)

V(9M) - 8, VL  $\approx$  30, ex  $\approx$  300, LC (10  $\mu$ l) - 0



EXHIBIT

D94

6/5/97

18 ex minipreps  $\Rightarrow$  1-8, 10, 11 - look like rectis  
(~~correct~~ <sup>correct</sup> MW)

1) Ladder

2)  $\downarrow$  18 ex (1-12)

Take 1-8, 10, 11.

1) Ladder

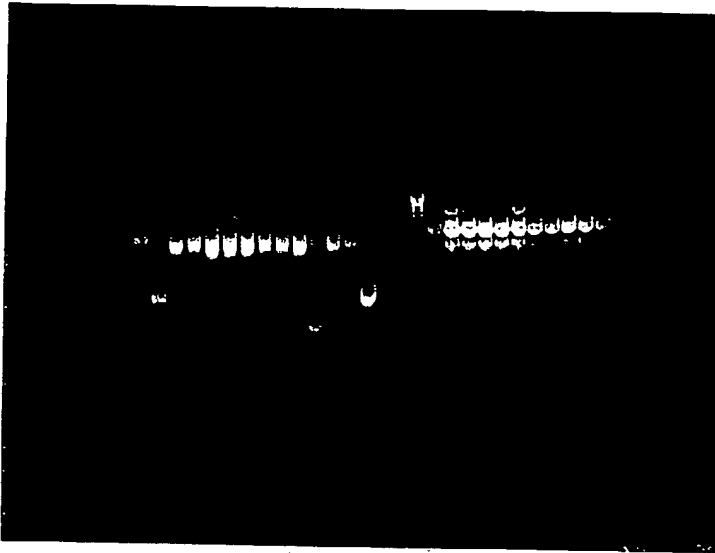
2) pCDNA3.1Zeo(+)

3)  $\downarrow$  18 ex  
1-8, 10, 11

Bam  
Xba

Look like rectis.

Grow 18(1).



22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS



EXHIBIT

tabbles

D95

6/5/97

VIPA - Infections:

Stock: ~~1101/1107~~, 1101/1107, 544, 101-1, 111-1, 734.1

E - egg (9-15)  
L - late (24-28)

(295)E	(293-1)E	(A549)E	(295)L	(293)TE	(A529)L
M - 1					
1101/1107 - 2	7	13	19	26	36
544 - 3	8	14	23	26	32
101-1 - 4	9	15	26	27	33
111-1 - 5	10	16	28	28	34
734.1 - 6	11	17	23	24	35
	12	18	24	36	36

A549

1101/1107 - 100μl  
544 - 100μl  
101 - 1 - 100μl  
111 - 2 - 150μl  
734.1 - 15μl

295

1101/1107 - 100μl  
544 - 1μl  
101 - 1 - 100μl  
111 - 2 - 500μl  
734.1 - 10μl

- Viruses dosage  
(and stocks  
of viruses)



6/10/97  
Restriction pattern  
for 181 max prep.

- 1) Cadder
- 2) 181/Spelt + Pse1
- 3) 181/Spelt + Xho1

Pattern is as  
expected.

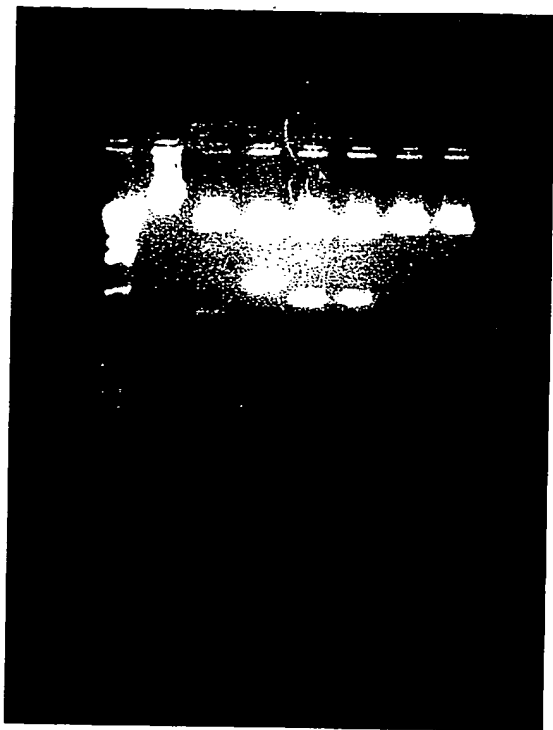
EXHIBIT

D96

ebbles

6/25/97

Restriction digestion of pCI-14.7 (Terry)



1) ladder

2) pCI-14.7

3) — EcoRI

4) — BglII

5) — SpeI + XhoI

6) — SpeI + SalI

7) — BamHI + XhoI

8) — BamHI + SalI

7/1/97 Clonings 19 ex; 20 ex.

19 ex — vector p#30 (SmaBI); insert

19 ex 1 { p181  
mfe  
XbaI  
Klenow

19 ex 2 { p181  
mfe  
RV  
Klenow

20 ex — vector pL2/PacI + T4; insert

20 ex 1 p181  
MX KL

7/3 transformation M52.

20 V ~ 10  
20 VL ~ 50  
20 ex 1 ~ 100  
20 ex 11 ~ 30

13 V ~ 20  
19 VL ~ 100  
19 ex 1 ~ 100  
19 ex 11 ~ 100

20 ex 2 p181  
M.R.V.KL

EXHIBIT

tabbles

D97





Dear Dan:

Attached is a list of primers that were ordered by Konstantin Doronin. The primers were named KD1, KD2, KD3, KD4, KD5, etc. (see Description/Sequences). These primers were used in studies on the ADP-overexpressing vectors, either for DNA sequencing or for amplification of certain sequences by the polymerase chain reaction (PCR). Also attached are Dr. Doronin's handwritten notes indicating the use of the primers. (These notes are not dated, but this list was begun when the primers were ordered). Please note that the primers were ordered on 9/26/96, 11/04/96, and 03/24/97.

Also included are some actual DNA sequence data using some of these primers to sequence some of the plasmids that were used to construct the ADP-overexpressing vectors. The dates of the sequencing runs are on the datasheets. Note that the first sequencing run was on November 19, 1996. Other runs were on November 25/26, 1996, March 20/21, 1997, April 21, 1997, May 12, 1997, and March 23/24, 1999.

Thanks,

Bill

Order Number		
Please refer to this No. on all Inquiries	Order Date	Page No.
473935 A	09/26/98	1 of 1

**SOLD TO**

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DEPT OF MICROBIOLOGY  
1402 S GRAND BLVD  
ST LOUIS, MO 63104  
DORONIN  
000127752

MLR	5003851		FAX/DEBBIE HUMISTON
-----	---------	--	---------------------

1.0	10336-022	KD1 \ DORONIN	1 Each	( G07 )	19.3
	A3884G07	CTACGAGAGAACCTCTCCGAG			
2.0	10336-022	KD2 \ DORONIN	1 Each	( G08 )	15.5
	A3884G08	GCCACAACCTTATACTGTTTGC			
3.0	10336-022	KD3 \ DORONIN	1 Each	( G09 )	28.6
	A3884G09	TCAGCCCACGGTACTTAATTAACCCAAAAGGTGGAT			
4.0	10336-022	KD4 \ DORONIN	1 Each	( G10 )	18.6
	A3884G10	GCTCTAGAAGTCAGGCTTCCTGG			
5.0	10336-022	KD5 \ DORONIN	1 Each	( G11 )	17.9
	A3884G11	GCTCTAGATCTCATTTAATCATA			
6.0	10336-022	KD6 \ DORONIN	1 Each	( G12 )	21.5
	A3884G12	CCTTAATTAAAAGTCAGGCTTCCTGG			
7.0	10336-022	KD7 \ DORONIN	1 Each	( H01 )	20.3
	A3884H01	CCTTAATTAATCTCATTTAATCATA			
8.0	10336-022	KD8 \ DORONIN	1 Each	( H02 )	21.7
	A3884H02	CGCCTATACAGAAGATTTTCCAG			
9.0	10336-022	KD9 \ DORONIN	1 Each	( H03 )	20.5
	A3884H03	CGCCTATACACTGCAGCAGGTGTG			

EXHIBIT

B1

**GIBCO BRL Custom Primers**  
*Certificate of Analysis*

ST LOUIS UNIV SCHOOL OF
Order Number: 473935A
Order Date: 09/26/96

**Primer 1:**

Primer Name: KD1  
Researcher: DORONIN  
Sequence (5' to 3'): CTA CGA GAG AAC CTC TCC GAG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6740.2  
Millimolar Extinction Coefficient: 230.5  
**Purity** **Deprotected**  
Tm (1 M Na+) 73  
Tm (50 mM Na+) 51  
% GC 57

Primer Number: A3884G07 (G07)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 29.2  
nmoles per OD: 4.3  
OD's 19.3  
 $\mu\text{g}$ 's\* 566  
nmoles 84  
Coupling Eff. 99%

Notes:



**Primer 2:**

Primer Name: KD2  
Researcher: DORONIN  
Sequence (5' to 3'): GCC ACA ACT TAT ACT GTT TGC  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6696.2  
Millimolar Extinction Coefficient: 221.4  
**Purity** **Deprotected**  
Tm (1 M Na+) 67  
Tm (50 mM Na+) 45  
% GC 42

Primer Number: A3884G08 (G08)  
Primer Length: 21  
 $\mu\text{g}$  per OD: 30.2  
nmoles per OD: 4.5  
OD's 15.5  
 $\mu\text{g}$ 's\* 471  
nmoles 70  
Coupling Eff. 99%

Notes:

**Primer 3:**

Primer Name: KD3  
Researcher: DORONIN  
Sequence (5' to 3'): TCA GCC CAC GGT ACT TAA TTA ACC CAA AAG GTG  
GAT  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 11632.2  
Millimolar Extinction Coefficient: 407.2  
**Purity** **Deprotected**  
Tm (1 M Na+) 81  
Tm (50 mM Na+) 59  
% GC 44

Primer Number: A3884G09 (G09)  
Primer Length: 36  
 $\mu\text{g}$  per OD: 28.5  
nmoles per OD: 2.4  
OD's 28.6  
 $\mu\text{g}$ 's\* 817  
nmoles 70  
Coupling Eff. 99%

Notes:

\* -See Note about Quantities in  
Supporting Information.

**GIBCO BRL Custom Primers**  
**Certificate of Analysis****ST LOUIS UNIV SCHOOL OF****Order Number: 473935A****Order Date: 09/26/96****Primer 4:**

Primer Name: KD4  
Researcher: DORONIN  
Sequence (5' to 3'): GCT CTA GAA GTC AGG CTT CCT GG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7429.6  
Millimolar Extinction Coefficient: 244.0  
Purity Deprotected  
Tm (1 M Na+) 75  
Tm (50 mM Na+) 54  
% GC 56

Primer Number: A3884G10 (G10)  
Primer Length: 23  
 $\mu\text{g}$  per OD: 30.4  
nmoles per OD: 4.1  
OD's 18.6  
 $\mu\text{g's}^*$  568  
nmoles 76  
Coupling Eff. 99%

Notes:

**Primer 5:**

Primer Name: KD5  
Researcher: DORONIN  
Sequence (5' to 3'): GCT CTA GAT CTC ATT TAA TCA TA  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7346.6  
Millimolar Extinction Coefficient: 251.4  
Purity Deprotected  
Tm (1 M Na+) 65  
Tm (50 mM Na+) 43  
% GC 30

Primer Number: A3884G11 (G11)  
Primer Length: 23  
 $\mu\text{g}$  per OD: 29.2  
nmoles per OD: 3.9  
OD's 17.9  
 $\mu\text{g's}^*$  523  
nmoles 71  
Coupling Eff. 99%

Notes:

**Primer 6:**

Primer Name: KD6  
Researcher: DORONIN  
Sequence (5' to 3'): CCT TAA TTA AAA GTC AGG CTT CCT GG  
Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 8370.2  
Millimolar Extinction Coefficient: 284.9  
Purity Deprotected  
Tm (1 M Na+) 73  
Tm (50 mM Na+) 51  
% GC 42

Primer Number: A3884G12 (G12)  
Primer Length: 28  
 $\mu\text{g}$  per OD: 29.3  
nmoles per OD: 3.5  
OD's 21.5  
 $\mu\text{g's}^*$  634  
nmoles 76  
Coupling Eff. 99%

Notes:

\* - See Note about Quantities in  
Supporting Information.

**LIFE TECHNOLOGIES**

# GIBCO BRL Custom Primers Certificate of Analysis

ST LOUIS UNIV SCHOOL OF

Order Number: 473935A

Order Date: 09/26/96

**Primer 7:**

Primer Name: KD7  
 Researcher: DORONIN  
 Sequence (5' to 3'): CCT TAA TTA ATC TCA TTT AAT CAT A  
 Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7957.0  
 Millimolar Extinction Coefficient: 277.0  
 Purity Deprotected  
 Tm (1 M Na<sup>+</sup>) 63  
 Tm (50 mM Na<sup>+</sup>) 41  
 % GC 20

Primer Number: A3884H01 (H01)  
 Primer Length: 25  
 $\mu\text{g}$  per OD: 28.7  
 nmoles per OD: 3.6  
 OD's 20.3  
 $\mu\text{g's}^*$  585  
 nmoles 73  
 Coupling Eff. 99%

Notes:

**Primer 8:**

Primer Name: KD8  
 Researcher: DORONIN  
 Sequence (5' to 3'): CGC GTA TAC AGA AGA TTT TTC CAG  
 Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7742.8  
 Millimolar Extinction Coefficient: 268.2  
 Purity Deprotected  
 Tm (1 M Na<sup>+</sup>) 70  
 Tm (50 mM Na<sup>+</sup>) 49  
 % GC 41

Primer Number: A3884H02 (H02)  
 Primer Length: 24  
 $\mu\text{g}$  per OD: 28.8  
 nmoles per OD: 3.7  
 OD's 21.7  
 $\mu\text{g's}^*$  628  
 nmoles 81  
 Coupling Eff. 99%

Notes:

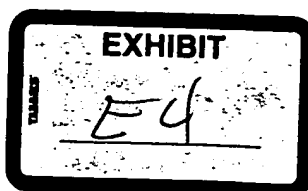
**Primer 9:**

Primer Name: KD9  
 Researcher: DORONIN  
 Sequence (5' to 3'): CGC GTA TAC ACT GCA GCA GGT GTG  
 Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7784.8  
 Millimolar Extinction Coefficient: 261.8  
 Purity Deprotected  
 Tm (1 M Na<sup>+</sup>) 77  
 Tm (50 mM Na<sup>+</sup>) 56  
 % GC 58

Primer Number: A3884H03 (H03)  
 Primer Length: 24  
 $\mu\text{g}$  per OD: 29.7  
 nmoles per OD: 3.8  
 OD's 20.5  
 $\mu\text{g's}^*$  609  
 nmoles 78  
 Coupling Eff. 99%

Notes:

\* See Note about Quantities in  
 Supporting Information.



LIFE  TECHNOLOGIES

Order Number		
Please refer to this No. on all Inquiries	Order Date	Page No.
522393 A	11/04/96	1 of 1

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ST LOUIS, MO 63104  
000127752

ORDER TYPE	CUSTOMER PHONE	CUSTOMER FAX	ORDER NO.	ORDER DATE	ORDER TIME
CEH	S903851				
FAX/DEBBIE HUMISTON					

1.0	10336-022	KD 10 \ DORANIN	1 Each	( B02 )	12.6
	A4379B02	ACACGGCACCAGCTCAATCAG			
2.0	10336-022	KD 11 \ DORANIN	1 Each	( B03 )	12.5
	A4379B03	CGGAGTAACTTGATGTGTTG			

**EXHIBIT**

ES

# GIBCO BRL Custom Primers Certificate of Analysis

ST LOUIS UNIV SCHOOL OF

Order Number: 522393A

Order Date: 11/04/96

**Primer 1:**

Primer Name: KD 10

Researcher: DORANIN

Sequence (5' to 3'): ACA CGG CAC CAG CTC AAT CAG

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6709.2

Millimolar Extinction Coefficient: 232.1

Purity Deprotected

Tm (1 M Na+) 73

Tm (50 mM Na+) 51

% GC 57

Notes:

Primer Number: A4379B02 (B02)

Primer Length: 21

 $\mu\text{g}$  per OD: 28.9

nmoles per OD: 4.3

OD's 12.6

 $\mu\text{g's}^*$  366

nmoles 54

Coupling Eff. 98%

**Primer 2:**

Primer Name: KD 11

Researcher: DORANIN

Sequence (5' to 3'): CGG AGT AAC TTG TAT GTG TTG

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 6847.2

Millimolar Extinction Coefficient: 233.0

Purity Deprotected

Tm (1 M Na+) 67

Tm (50 mM Na+) 45

% GC 42

Notes:

Primer Number: A4379B03 (B03)

Primer Length: 21

 $\mu\text{g}$  per OD: 29.3

nmoles per OD: 4.2

OD's 12.5

 $\mu\text{g's}^*$  369

nmoles 53

Coupling Eff. 98%

\* -See Note about Quantities in  
Supporting Information.

LIFE  TECHNOLOGIES™

Order Number		
Please refer to this No. on all Inquires	Order Date	Page No.
409887 A	03/24/97	1 of 1

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DEBBIE HUMISTON  
000127752

CUSTOMER REF	CUSTOMER PAYING	CUSTOMER CONTRACT NO	RELEASE NO	PLACED BY
CD	S903850			DEBBIE HUMISTON
ITEM NO	QTY	DESCRIPTION	UNIT PRICE	AMOUNT

1.0	10336-022	KD12 \ DORONIN	1 Each	( D10 )	16.4
	A6280D10	CCTTAATTAATCTAGAGATCTTATCCCTTT			
2.0	10336-022	KD14 \ DORONIN	1 Each	( D11 )	14.7
	A6280D11	GGGGTACGAAGCCATCTGCAACAACAT			
3.0	10336-022	DK15 \ DORONIN	1 Each	( D12 )	13.7
	A6280D12	CCTTAATTAATCTAGAGTCAGTTAGCCTCCCC			
4.0	10336-022	KD16 \ DORONIN	1 Each	( E01 )	14.6
	A6280E01	CGCGCGTATACACTTCCCATTTTAAG			
5.0	10336-022	KD17 \ DORONIN	1 Each	( E02 )	12.9
	A6280E02	GCTCTAGACATCATCAATAATAT			





ST LOUIS UNIV SCHOOL OF

Order Number: 409867A

Order Date: 03/24/97

# GIBCO BRL Custom Primers

## Certificate of Analysis

**Primer 4:**

Primer Name: KD18

Researcher: DORONIN

Sequence (5' to 3'): CGC GCG TAT ACA CTT CCC ATT TTA AG

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 8306.2

Millimolar Extinction Coefficient: 272.8

Purity Deprotected

Tm (1 M Na+) 74

Tm (50 mM Na+) 53

% GC 46

Notes:

Primer Number: A6280E01 (E01)

Primer Length: 28

 $\mu\text{g}$  per OD: 30.4

nmoles per OD: 3.6

OD's 14.5

 $\mu\text{g's}^*$  444

nmoles 53

Coupling Eff. 99%

**Primer 5:**

Primer Name: KD17

Researcher: DORONIN

Sequence (5' to 3'): GCT CTA GAC ATC ATC AAT AAT AT

Molecular Weight  $\mu\text{g}/\mu\text{mole}$ : 7364.6

Millimolar Extinction Coefficient: 263.4

Purity Deprotected

Tm (1 M Na+) 66

Tm (50 mM Na+) 43

% GC 30

Notes:

Primer Number: A6280E02 (E02)

Primer Length: 23

 $\mu\text{g}$  per OD: 27.9

nmoles per OD: 3.8

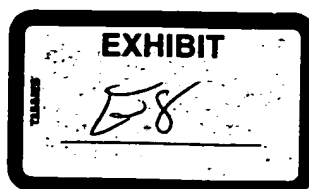
OD's 12.9

 $\mu\text{g's}^*$  361

nmoles 49

Coupling Eff. 99%

\*-See Note about Quantities in  
Supporting Information.

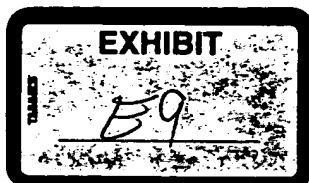
LIFE  TECHNOLOGIES.

## Primers:

1) KD1 - CTA'CGA'GAG'AAC'CTC'TCC'GAG'

Comment - primer from AdS sequence ~ 50 nt left from SmaI site -  
for PCR mutation of gp13K (E3) - Left primer.

2) KD2 - GCC'ACA'ACT'TAT'ACT'GTT'TGC

Comment - primer from AdS sequence ~ 50 nt right from Bst 1107I site -  
for PCR mutation of gp13K (E3) - right primer.3) KD3 - TCA'GCC'CAC'GGT'ACT'T(TAA)TTAACC'CAA'AAG'GTG'GAComment - middle primer for mutation of gp13K E3 protein gene.  
inserting PacI site with TAA stop codon to the ORF  
destroying KpnI site simultaneously, to be used 1 with KD2,  
then product used as primer with KD1.4) KD4 - GC'TCTAGA'AGT'CAG'GCT'TCC'TGGComment - left primer for PCR of 11.6K E3 with XbaI site.5) KD5 - GC'TCTAGA'TCT'CAT'TTA'ATC'ATAComment - right primer for PCR of 11.6K E3 with XbaI site.6) KD6 - CC'TTAATTAA'GT'CAG'GCT'TCC'TGGComment - left primer for PCR of 11.6K E3 with PacI site7) KD7 - CC'TTAATTAA'TCT'CAT'TTA'ATC'ATAComment - right primer for PCR of 11.6K E3 with PacI site.8) KD8) CGC'GTATAC'AGA'AGA'TTT'TTC'CAG'upstream primer with Bst 1107I site for PCR of SP-B 500 promoter.9) KD9) CGC'GTATAC'ACT'GCA'GCA'GGT'GTCdownstream primer with Bst 1107I site for PCR of SP-B 500 promoter.22-141 50 SHEETS  
22-142 100 SHEETS  
22-144 200 SHEETS

Primers for sequencing  $\phi$ E4 substitutions ;  
primer.

- 10) K1 (10): ACA'CGG'CAC'GAG'CTC'AAT'GAG' (left)  
11) K1 (11): CCG'AGT'AAC'TTG'TAT'GTG'TTG (right)

Primers for TIC insert into Pac and Xba sites. (double PCR,  
12+14, then product with 15  
on Ad template).

CC  
~~CC~~ TTAATTA TCTAGA GAT'CTT'ATT'CCC'TTT

Primer with PacI and XbaI sites + Ad seq 30818  $\rightarrow$   
upstream primer.

14) K1 (14)  $\leftarrow$  left

GGG'GTA'CGA'AGC'CAT'CTG'CAA'CAA'CAT

Primer left half from 5' half, right half - from Ad ~ 31000  
 $\Rightarrow$  beginning of fiber gene. to prevent methylation.

15) K1 (15) CC TTAATTAA TCTAGA G TCA'CTT'AGC'CTC'CCC  
 $\leftarrow$  right

Primers for PCR Ad5 right ITR (Ad. 35773-35935) with  
flanking bst1107I and XbaI sites. respectively.

16) K1 (16) CCGCCGTATAC ACTITCC'CAT'TTT'AAG

17) K1 (17) GCTCTAGA CAT'CAT'CAA'TAA'TAT

18) (18) - Direct - GTA-GAG-TTT-TCT-CCT-CCG  
for PCR and sequence of 1101/1107 dl.

19) (19) - Reverse - CCC-TCT-TCA-TCC-TCG-TCG

20) TCT, ACT, TTA, ACC, CCA, TCT, CCG CE3 direct to. C1 d1)

21) CCG, AGG, TGT, TAT, TAC, CGA, AGA CE1A, M01 d1)

22) TGA, CGT, AAC, CCA, TAA, AGT, CCA (from E4 prim d1)  
direct

50 SHEETS  
22-141  
22-142 100 SHEETS  
22-144 200 SHEETS

EXHIBIT

E16

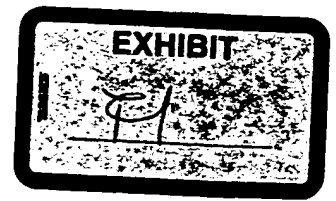
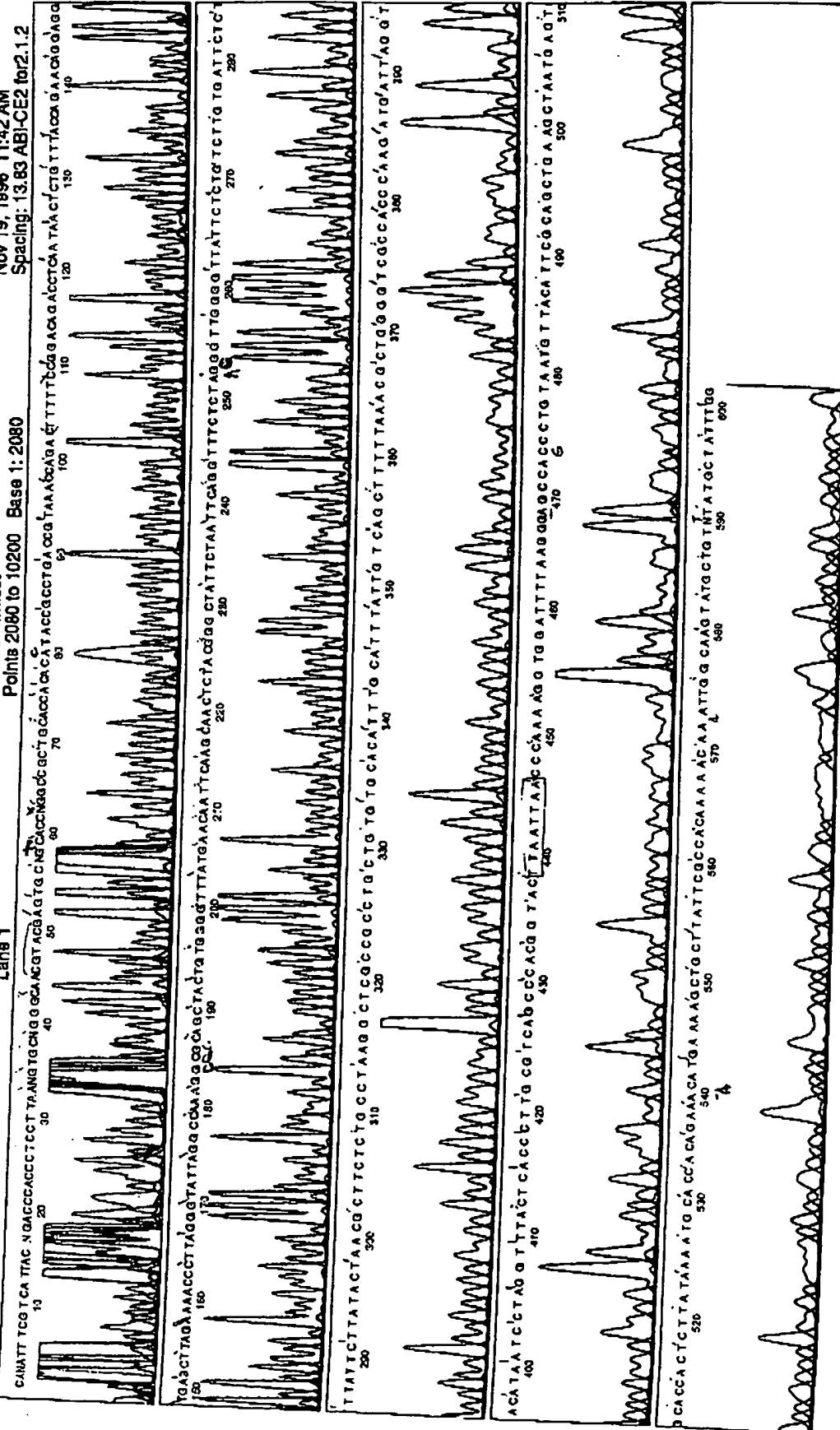
3ex Chrom 1  
A1-KD primer 1 (131)  
KD  
Lane 1

Model 310  
Version 2.12

ABI  
PRISM

Signal G:340 A:481 T:305 C:253  
DT POP6 10-10-96  
POP8DT.Matrx  
Points 2080 to 10200 Base 1: 2080

Page 1 of 1  
Nov 19, 1996 2:40 PM  
Nov 19, 1996 11:42 AM  
Spacing: 13.83 ABI-CE2 for 2.1.2





**Spacing: 13.29 ABICE2 for 2.12**





Model 310  
Version 2.1.2

A11-KD-#5

KD-#5  
Lane 8

1ex, clone 23, primer 1310.

Signal G:339 A:578 T:329 C:216

DT POP6 10-10-96

POP6DT.Matrix

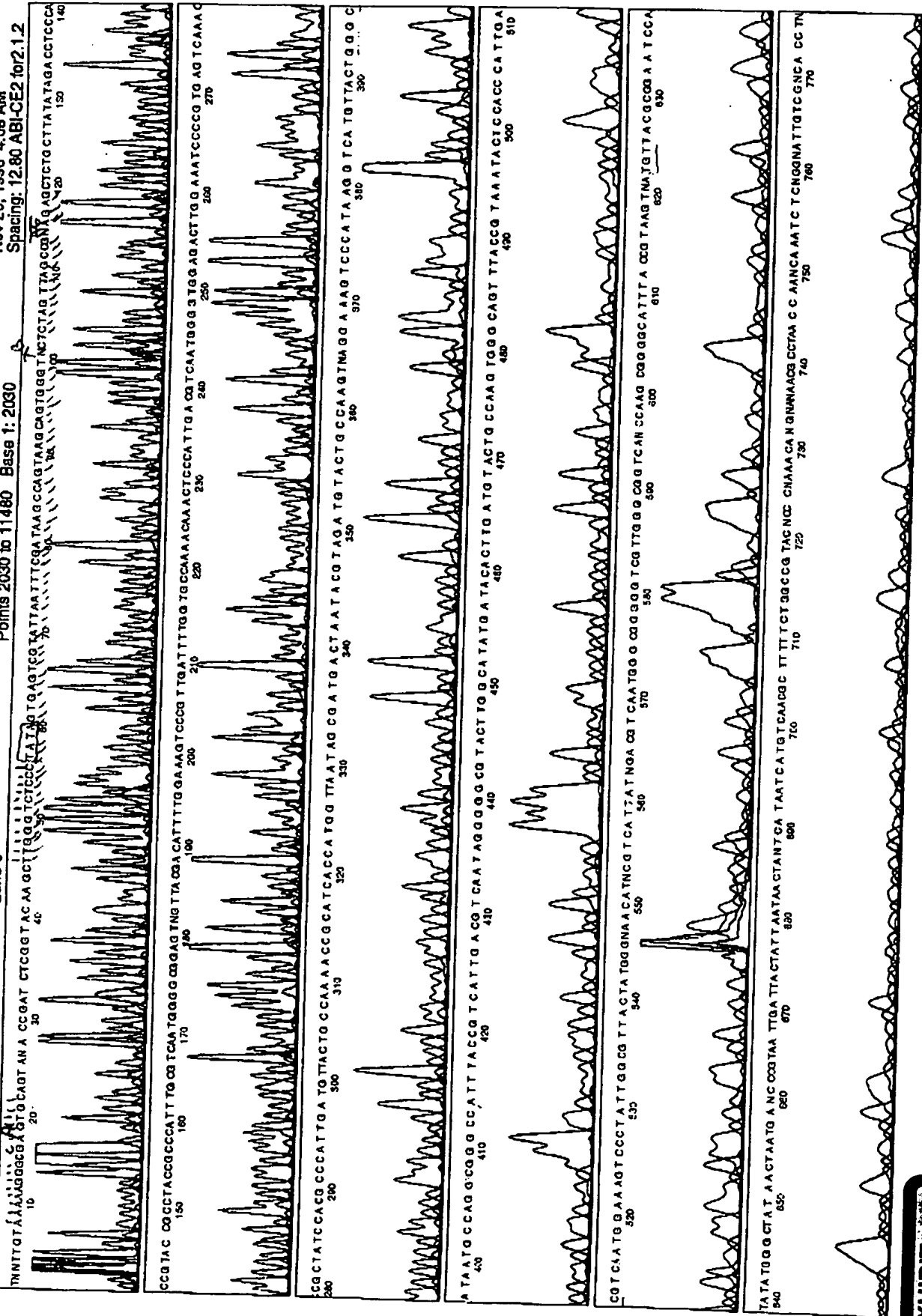
Points 2030 to 11480 Base 1: 2030

Page 1 of 2

Nov 26, 1996 8:53 AM

Nov 26, 1996 4:08 AM

Spacing: 12.80 ABI-CE2 for 2.1.2



EXHIBIT



Model 310  
Version 2.1.2**ABI**  
**PRISM**

B2-KD-#6

KD-#6  
Lane 7

Signal G:343 A:526 T:302 C:183

DT POP6 10-10-96

POP6DT.Matrx

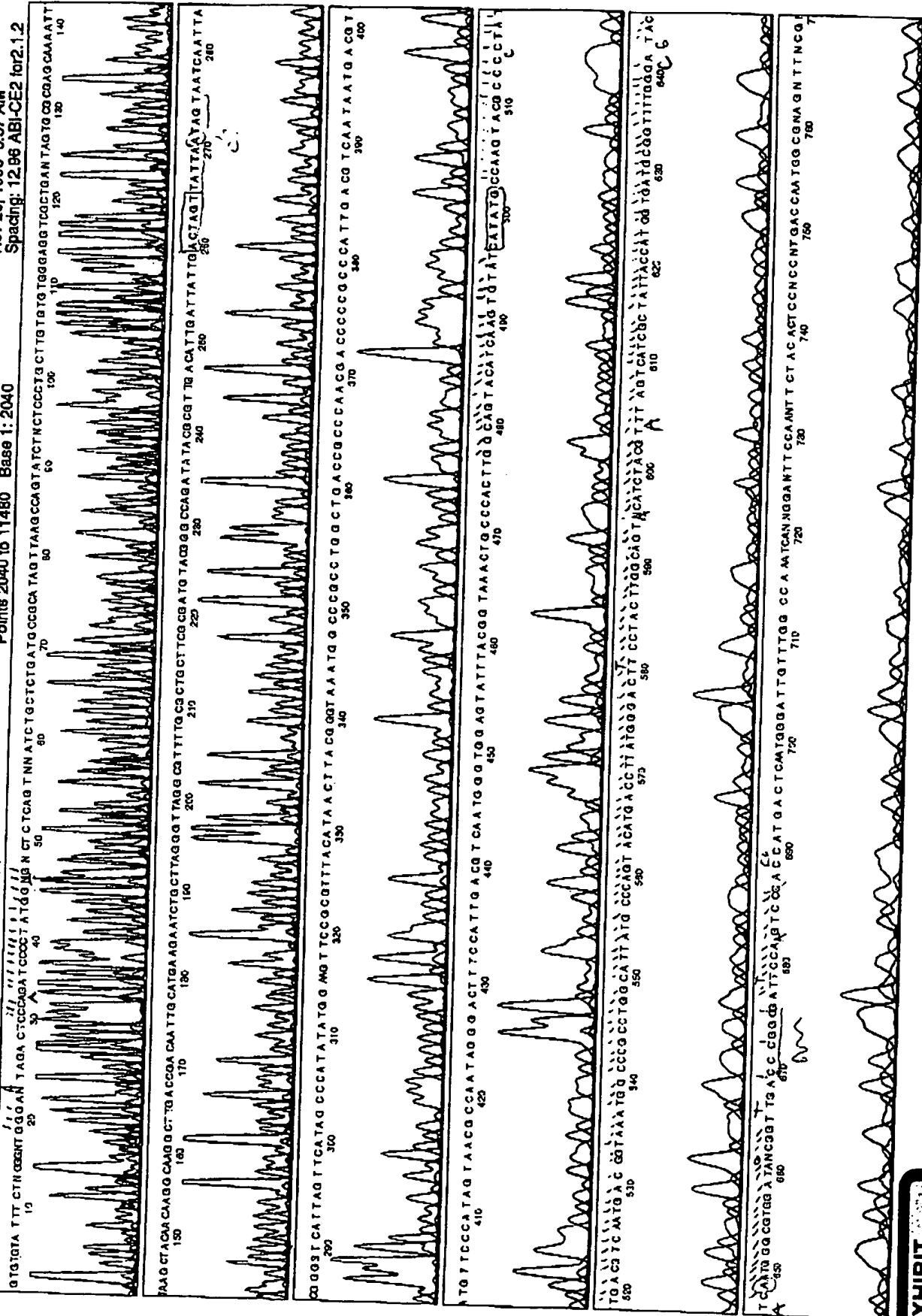
Points 2040 to 11480 Base 1:2040

Page 1 of 2

Nov 26, 1996 9:54 AM

Nov 26, 1996 6:57 AM

Spacing: 12.96 ABI-CE2 for 2.1.2

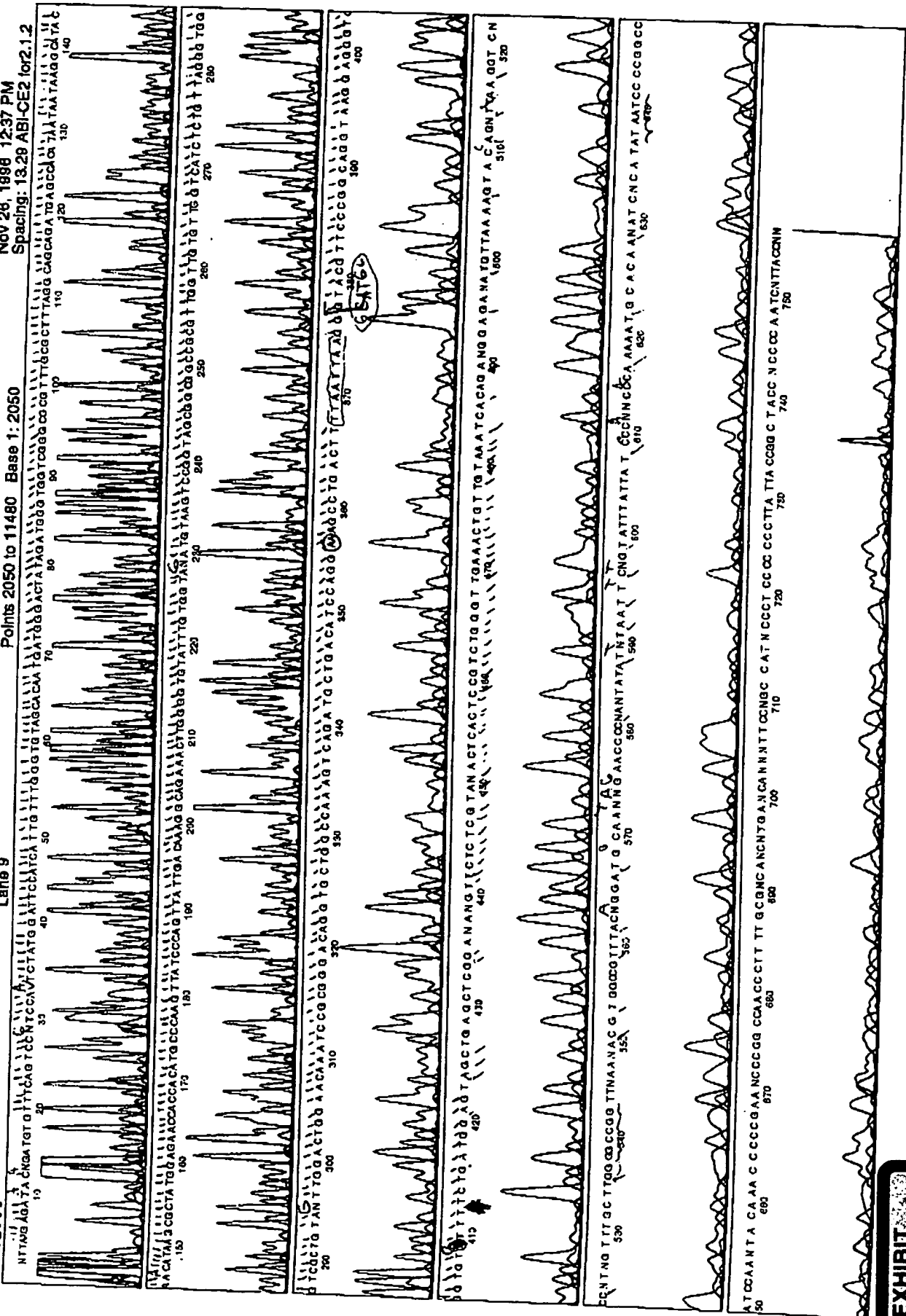


EXHIBIT

F6

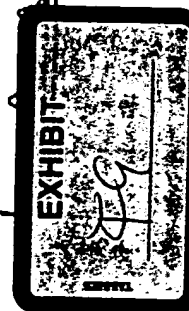


Nov 26, 1996 3:28 PM  
Nov 26, 1996 12:37 PM  
Spacing: 13.29 ABI-CE2 for 2.1.2



# EXHIBIT

Page 1 of 1  
Thu, Mar 20, 1997 7:47 PM  
Thu, Mar 20, 1997 5:02 PM  
Subject: 11-204-2001



WY 10  
(p21, primer 11)

ABI PRISM  
Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

A3-Wold-10  
Wold-10  
Lane 2

Signal G:839 A:658 T:543 C:294  
DT POP6  
pop6 di-septre-stds.mtx  
Points 1043 to 9400 Base 1: 1043

Page 1 of 1  
Thu, Mar 20, 1997 10:22 PM  
Thu, Mar 20, 1997 7:47 PM  
Spacing: 12.19(12.19)

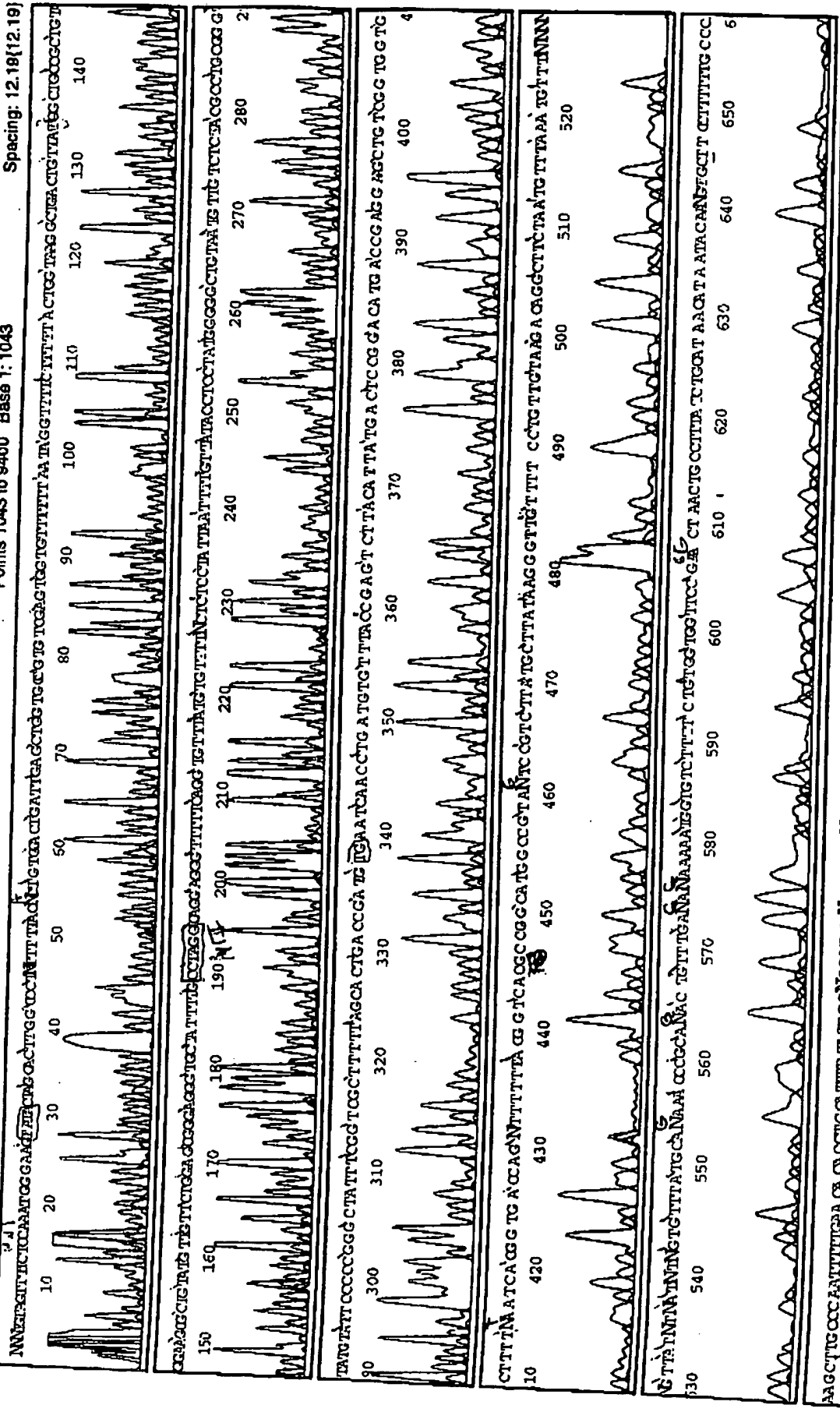


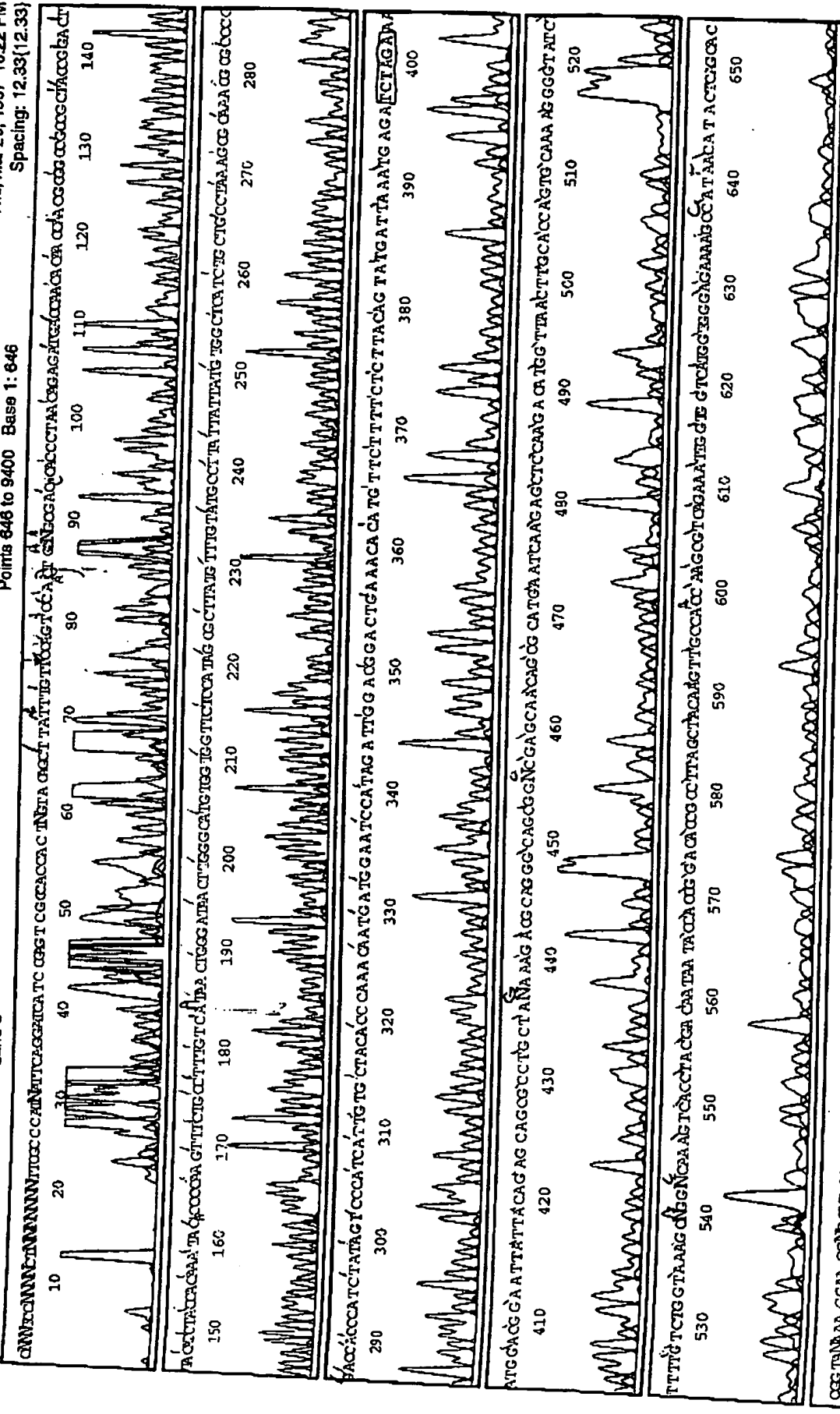
EXHIBIT  
1  
790

Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

AS-Wold-11  
Wold-11  
Lane 3

Signal G:278 A:413 T:211 C:190  
DT POP6  
pop6 dt-sep-stds.mtx  
Points 648 to 9400 Base 1: 646

Page 1 of 1  
Fri, Mar 21, 1997 12:57 AM  
Thu, Mar 20, 1997 10:22 PM  
Spacing: 12.33(12.33)



Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

A7-Wold-12  
Wold-12  
Lane 4

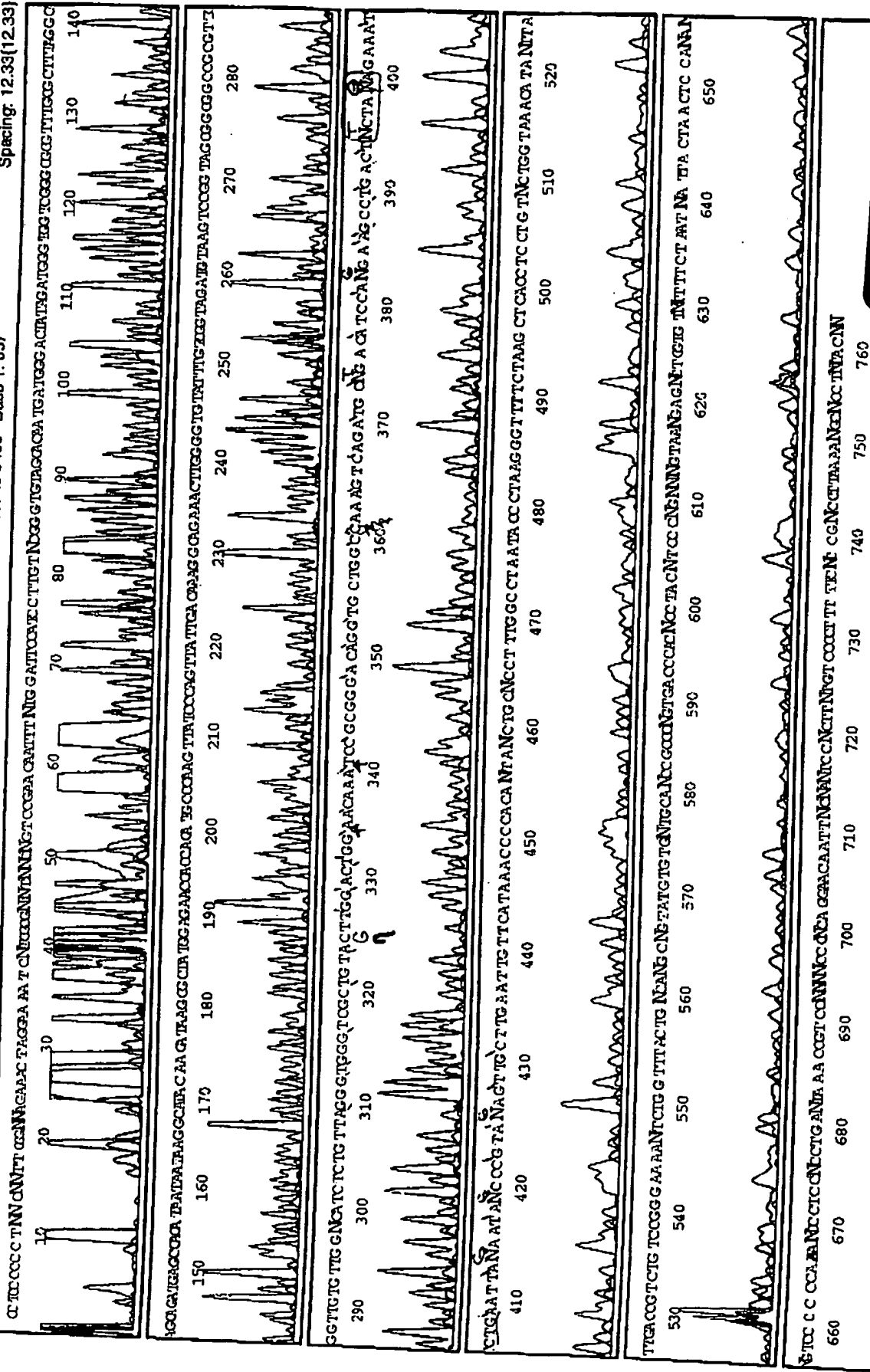
Signal G:251 A:255 T:180 C:128  
DT POP6  
pop6 dt-seprie-stds.mtx  
Points 657 to 9400 Base 1: 657

Page 1 of 1

Fri Mar 21, 1997 3:32 AM

Fri Mar 21, 1997 12:57 AM

Spacing: 12.33(12.33)



EXHIBIT

12/2

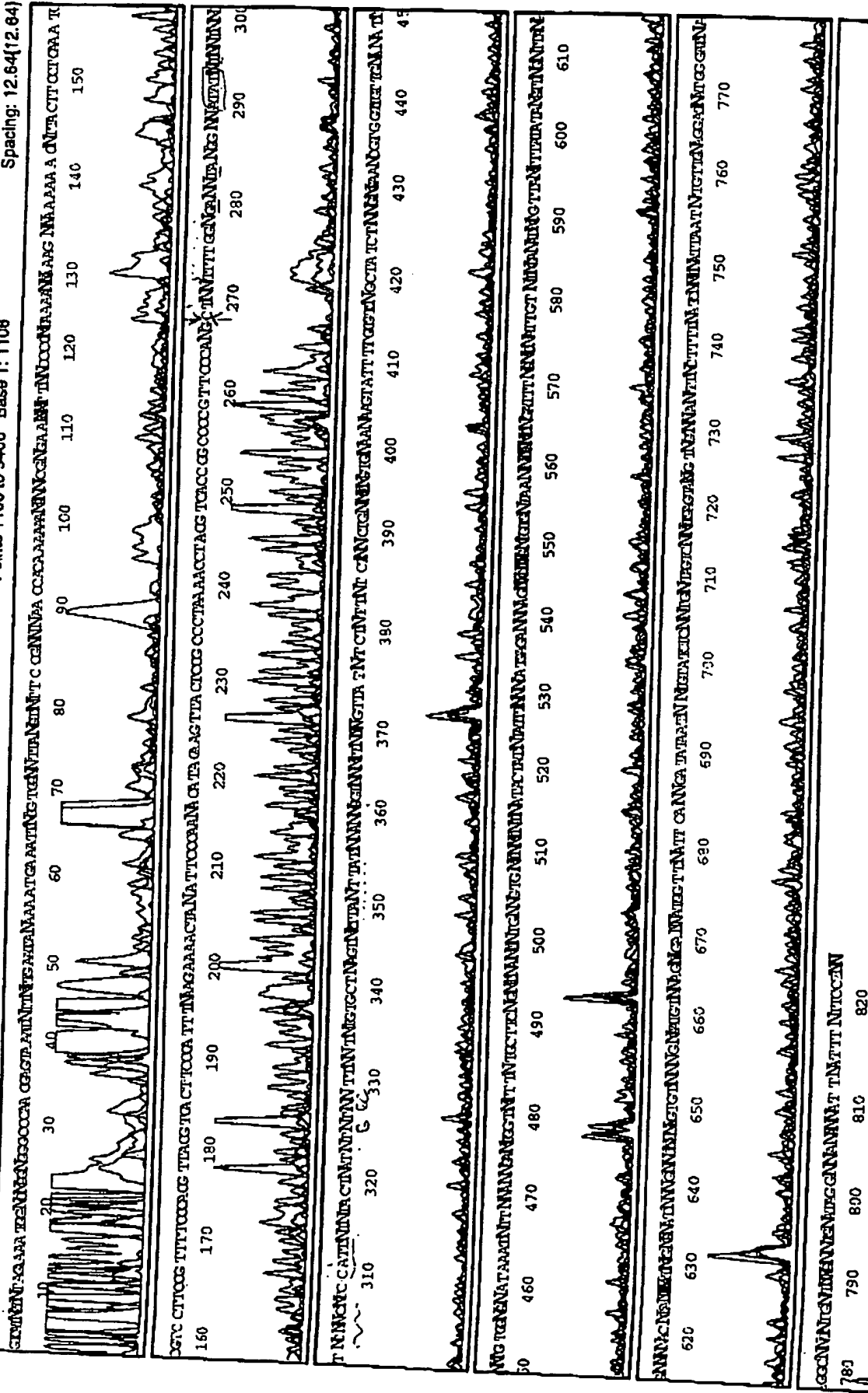
Model 310  
Version 3.0  
ABI-CE2 3.0  
Version 3.0b1

A1-Kostya/Wold-14  
Kostya/Wold-14  
Lane 1

Signal G:71 A:84 T:76 C:57  
DT POP8  
pop8 dt-sepirt-sids.mtx  
Points 1106 to 9400 Base 1: 1106

Page 1 of 1

Mon, Apr 21, 1997 12:49 PM  
Mon, Apr 21, 1997 9:22 AM  
Spacing: 12.84(12.84)

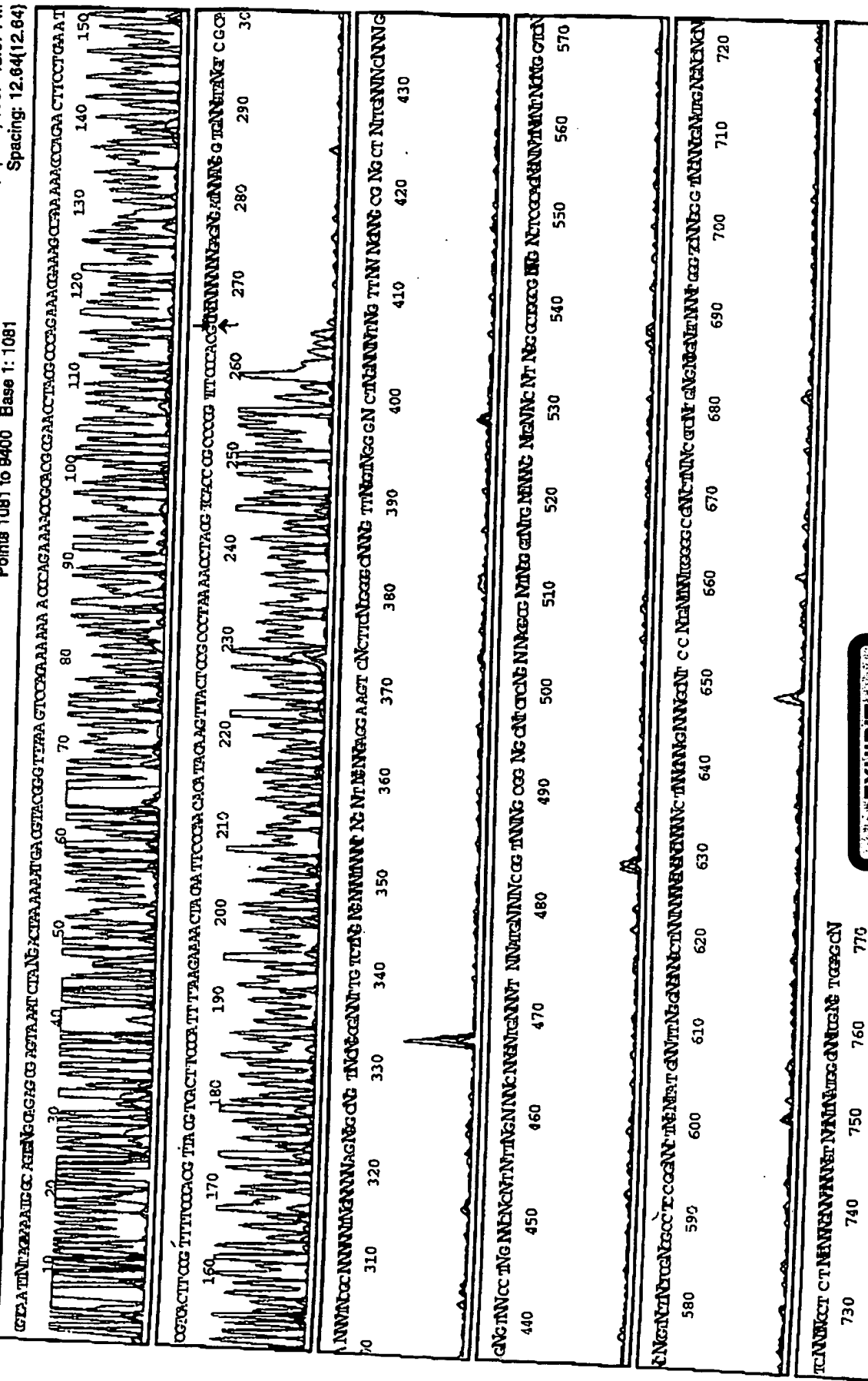




prince and io.

Signal G:151 A:330 T:175 C:161  
DT POP6  
pop6 dt-septe-stds.mlx  
Points 1081 to 8400 Base 1: 1081

Page 1 of 1  
Mon, Apr 21, 1997 2:42 PM  
Mon, Apr 21, 1997 12:07 PM  
Spacing: 12.64{12.64}



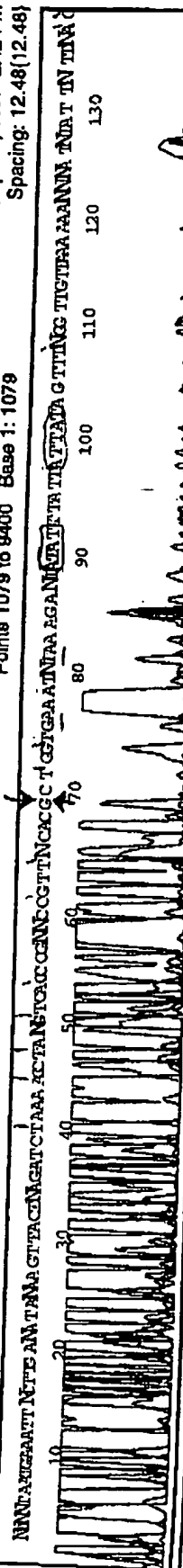
# EXHIBIT



Wagner's Early Large  
phased,  
minor 1916.

Points 1079 to 9400 Base 1:1079

Page 1 of 1  
Mon, Apr 21, 1997 5:18 PM  
Mon, Apr 21, 1997 2:42 PM  
Spacing: 12.48(12.48)



140	150	160	170	180	190	200	210	220	230	240	250	260	270
GA TTA TTTGGGCAAA NNNNNNTG TTNAGAGGGTCTA NNNNCA TG TA TTA CNG NNNNNNAGGCA NCGNATG TTTCA AATTCGGCT NNTA NNNNNGCTT GGTATT TG NNA TTTNAT TTTTAAAGNS													

280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	
AA NG	TTTNNNNNNNNNN	AAAA	ATT G	TCN	GGAT	TTTA	ATATA	AATTA	NTTTATATAT	TTTNG	TTTTTT	TTT	TTT	TTT	TTT
<p>Handwritten text: <i>Handwritten text is present across the top of the page, likely a header or title, but it is illegible due to the image quality.</i></p>															

[illegible][illegible]

20	730	740	750	760	770	780
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# EXHIBIT

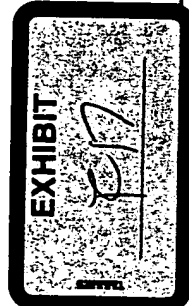
Handwritten signature

Page 1 of 1  
Mon, Apr 21, 1987 7:52 PM  
Mon, Apr 21, 1997 5:17 PM  
Spacing: 12.80(12.80)



**EXHIBIT**

Page 1 of 1  
Mon, Apr 21, 1997 10:27 PM  
Mon, Apr 21, 1997 7:52 PM  
Spacing: 12.48(12.48)





Wold/KD-#3(2-7)  
Lane 8

p142(1), printed by B

DT POP8

pop8 dt-sep18-stds.mtx

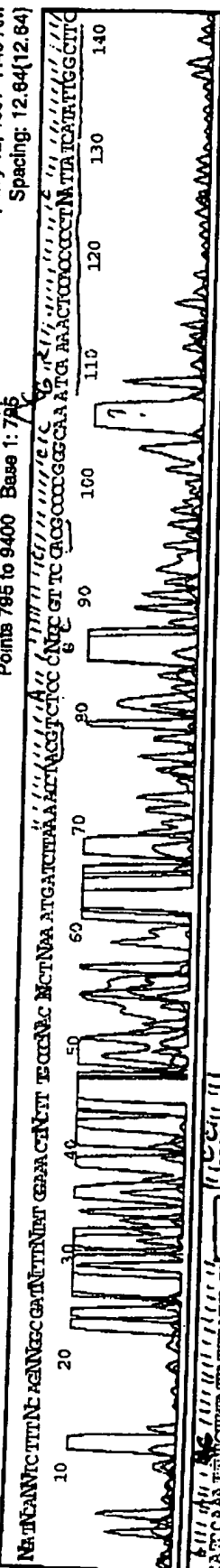
Points 795 to 9400 Base 1: 795

Page 1 of 1

Mon, May 12, 1997 10:24 AM

Mon, May 12, 1997 7:49 AM

Spacing: 12.84(12.84)



ATCAGA TT TGTGTTATTGATCA TCCTGGCGT GAGTAT TGC CTTTGG TGACTGTAATAA TTCCTGG AGCCATTTTAAACG CTGNG ACTGGGAAAAGC NCG NCTNNNCAATTTATATGCCCTTATTTTC

150 160 170 180 190 200 210 220 230 240 250 260 270 280

CATCCGCTTTGGCAGN7TGCTGTNA7ANCGAA GAGG CT CG TNNNE A TCGGNITTTTCNA A NAGG TGTT NNAGT TTNAAA TG G NV A ATG GGA NE CG CC TTNETTTTTNNNGS CA TTA ANINGTC

290 300 310 320 330 340 350 360 370 380 390 400 410

[illegible][illegible]

STATION	680	690	700	710	720	730	740
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EXHIBIT

12

Lane 8

**Model 310**  
**Version 3.0**  
**ABI-CE2 3.0**  
**Version 3.0b1**



Signal G:373 A:370 T:217 C:189

DT POP8

pop8 dt-sep18-stds.mtx

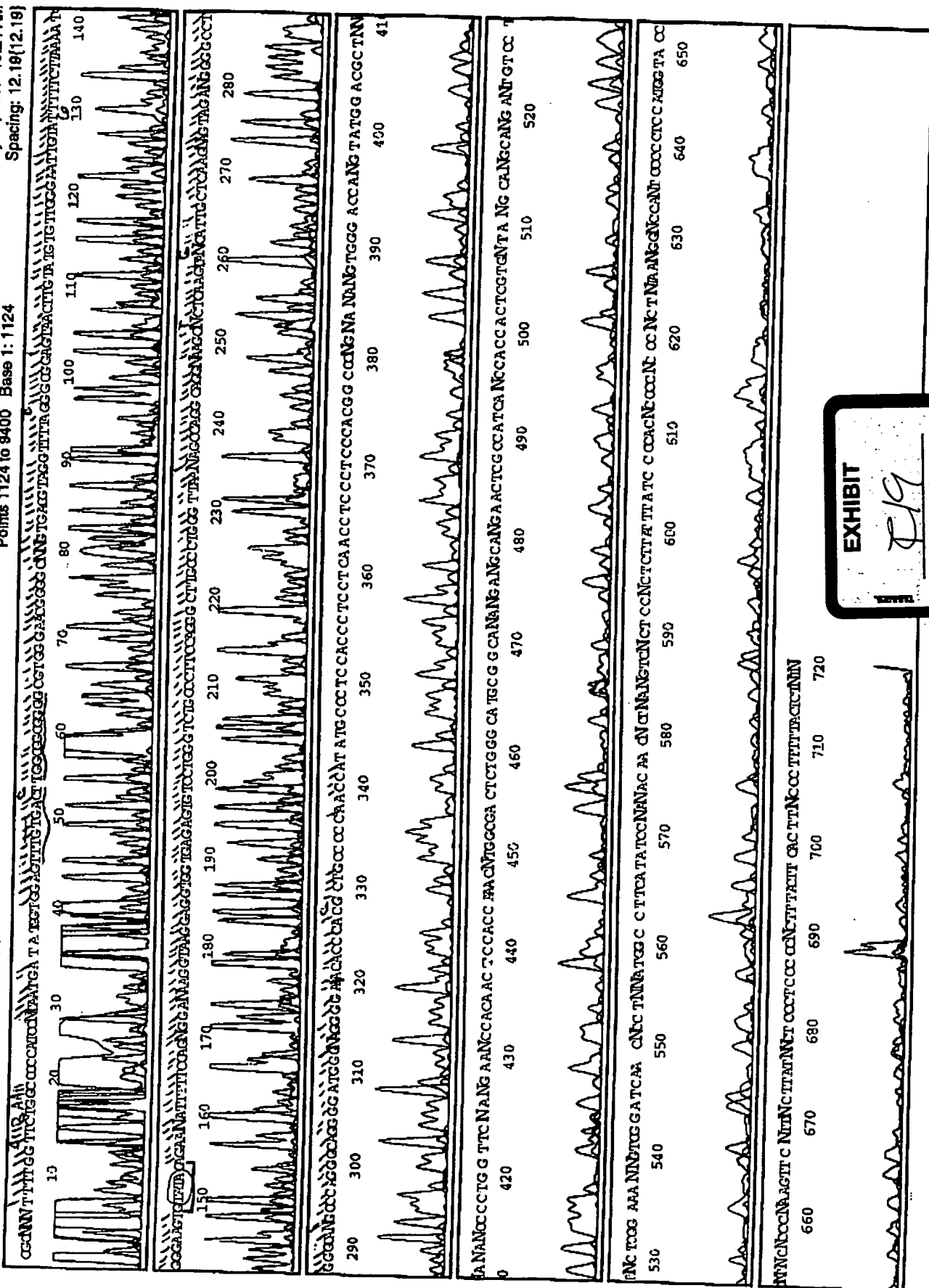
Points 1124 to 9400 Base 1:1124

Page 1 of 1

Mon, May 12, 1997 1:00 PM

Mon, May 12, 1997 10:24 AM

Spacing: 12.19{12.19}



Spacing: 12.12%

460

2

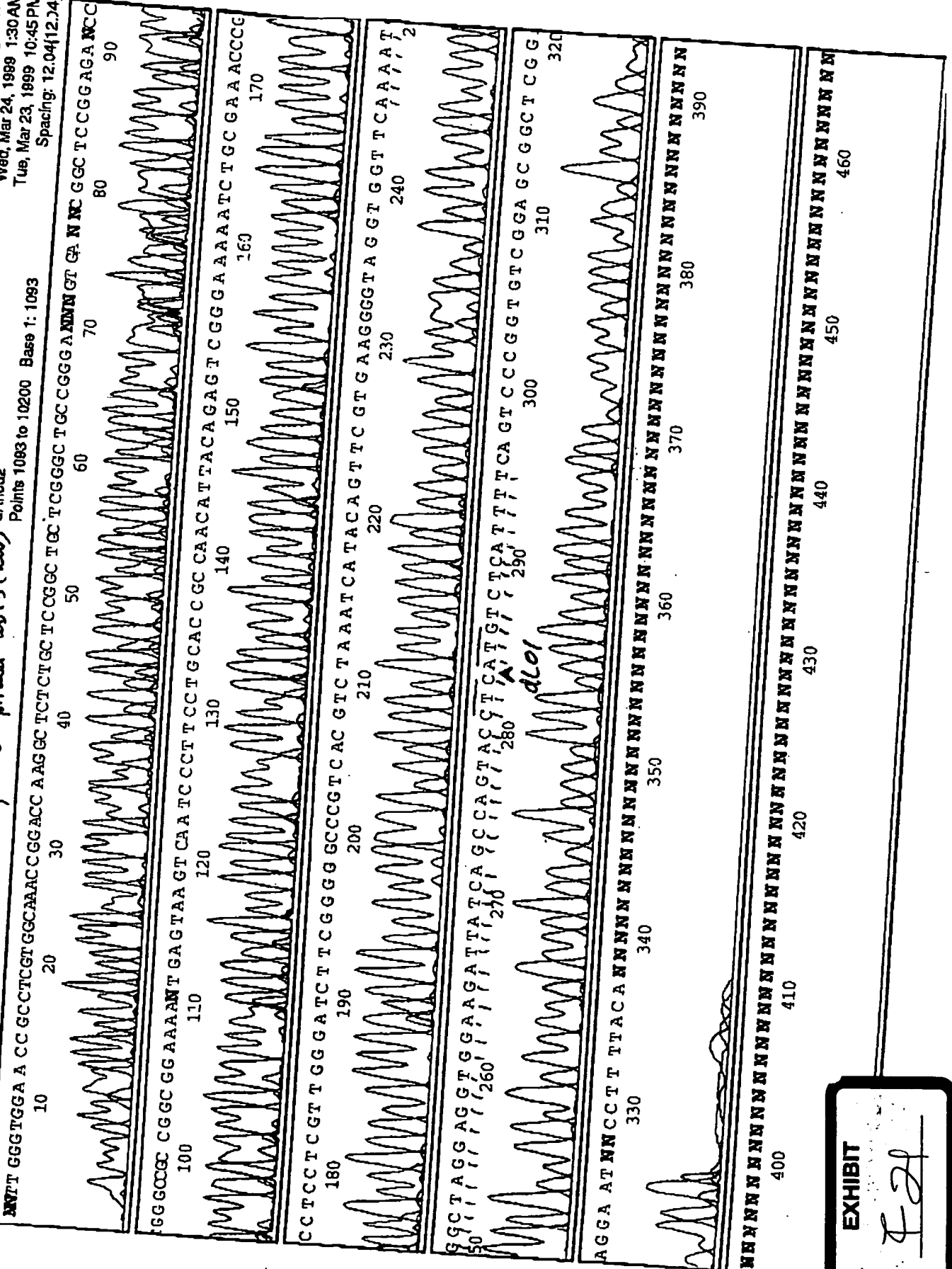
ABI  
PRISM<sup>+</sup>  
Model 310  
Version 3.0  
ABI-CE1  
Version 3.0

Doroin #2  
Doroin #2  
Lane 6

pel product  
1101/1107 43 (8-19)  
Sequence primer 43 (3 (rev))

Signal G:1919 A:1458 T:1194 C:1582  
DT POP6(BD Set-Any Primer)  
dRhod2  
Points 1083 to 10200 Base 1: 1093

Page 1 of 2  
Wed, Mar 24, 1989 1:30 AM  
Tue, Mar 23, 1989 10:45 PM  
Spacing: 12.04(12.04)



EXHIBIT

F-28







504

Page 1 of 2  
Mar 24, 1998 5:15 PM  
Mar 24, 1998 2:30 PM  
Spacing: 12.04(12.04)

Signal G:88 A:89 T:77 C:107  
DT POPE(BD Set-Any Primer)  
dRhod2  
Points 970 to 10200 Base 1:0

PL2, pinex 20 (D12)

Doronin #7  
Doronin #7  
Lane 2

Model 310  
Version 3.0  
ABI-CE1  
Version 3.0



NTTCTTTT NNGGACGG TTTA TTT TTTTATTTATTA CCGACTTTGAC CCGGTAAGGACTCGGCGGAC TCTTAC GACTGATGATTTTACTG NAGAGGCA GAGGACTGCG CTTGAATCAC C

10 20 30 40 50 60 70 80 90 100 110

Spacing: 12.04(12.04)

Percentage of the population aged 15 and over who are illiterate

1950 1960 1970

120 110 100 90 80 70 60 50 40 30 20 10 0

Algeria  
Congo  
Cote d'Ivoire  
Ghana  
Guinea  
India  
Indonesia  
Kenya  
Liberia  
Mali  
Mauritania  
Mauritius  
Morocco  
Niger  
Nigeria  
Senegal  
Sierra Leone  
Tanzania  
Togo  
Tunisia  
Uganda  
Zambia

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 2. 2. The second part of the book is a history of the  
 3. 3. The third part of the book is a history of the  
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 99. 99. The ninety-ninth part of the book is a history of the  
 100. 100. The hundredth part of the book is a history of the

GGT CAC TGT CGC CGC CACA GT GCT TTG CCG CGG ACT CCG GT GAG TTTT GCT ACT TT GA ATT GCC CGG AGG ATCA TAT C GAG GAT C TTT GT T GCC ATC

TGTGCTGAGTATNTAAATACAGAANTTAABATTACTCCCCCCTCCCTTC

230 240 250 260 270 280 290 300 310

Monday, 11/11/2019

AGG CGA A C C T T A C C T T A C A T C T C C C T C T G T A T T T A C A C A G T T T C A A C C C A G A C G G A G T G A G T C T A C G A G A C A C C C C C T A

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420 430 440 450 460 470 480  
 ...GTC TCA GC TACT CCA TCA T AAAA AACA CCACCC TCCTTACCTGCC GGAAC GTACCCCTTAA TTAAG GAA TT TC T GTCCA GTTT A TTCA GCAK

510	CT CCA GC TC TG GAT	TG C A A N T T N C T	C C T G C T T G C A A C	A T T T C T T C A C A A T	C T A A A T G G A A T G G	C A G T T T T C T C T G T T
520		530	540	550	560	570
530						
540						
550						
560						
570						
580						
590						
600						

pl 2, alkylne denaturation  
primer 6920 (Dir)

**ABI**  
**PRISM™**

Doronin #8  
Doronin #8  
Lane 3

Signal G:65 A:41 T:42 C:53  
DT POP6(BD Set-Any Prime)  
idRhod2  
Points 1076 to 10200 Base 1: 1076  
Wed, Mar 24, 1999 5:15 PM  
Spacing: 12.04(12.04)

CAGG GGGGCTATCC GGAAT AATTTT TTTCCATAC TTTTGGCC GGTAAAG GACTCCGGC GGAAGGCTTGAATTTTAACTGGAGAGGCA GAGCACTTGGCCCTGGAACACCTGGTCCAC  
 10 20 30 40 50 60 70ATC 80 90 100 110  
 2.5 mut. LKH  
 TGTGGCCGCCCAAGTGTCTTGGCCGCACTCCGGTCAAGTTTGGTACTTTGAATTGGCCGAGGATCATATC GAGGATCTTTGTATCCATCTCTGTGTCTC  
 120 130 140 150 160 170 180 190 200 210  
 LKH  
 GAGTATATATAAATACAGAAATATAA AATATATCTGGGGCTCC TATCTCCATCTCTGTAAACGCCACCTCTTCACCCCGCCCAAGCAACCAAGCCGAA  
 220 230 240 250 260 270 280 290 300 310  
 CTTACCTGGTACTTTTAAACATCTCTCCCTCTGTGATTATACAAAGGTTTCAACCCCAAGCGAATGAGTCTTACGAGAGAACCTNTCCGAGCTCAG  
 320 330 340 350 360 370 380 390 400  
 TATCTCRAATCAGAAATAACACCAACCTCTCTTACC TGC CGGGAACGTA CCGTTAAATTAAGGATTTCTGTCCATTTATTCANCAACACCTNCTTG  
 410 420 430 440 450 460 470 480 490 500  
 LKH at +pac insertion  
 NCCTCCTNCCANCTTNGGGTATTGCAACNTNCCCTCC TGNCTGCCAAACTTTCTCAGCAAA TCTAATNGAATGCAATNTTNTONTGTGNTONTCA T NCG  
 510 520 530 540 550 560 570 580 590 600

970619 Infection of A549 (Kostlyn's Virus)A549 (6/18) "1-40" — ~80% confl. (estimate  $10^6$  cells/TD)

Added SF-DME (2ml), removed &amp; added 1ml SF-DME (~11Am);

Infection at 2:30pm

<u>Virus</u>	<u>Prep</u>	<u>Titer</u>	<u>Inf'g Volume</u> (1:20 dilution)	<u># TD</u>
① mock	—	—	—	1
② dl. 309 (VS 119) (970308)	—	$1.2 \times 10^{11}$	10 $\mu$ l/TD	4
③ KD-1 <sup>(1101/1107)</sup> ADP (970508)	—	$9.64 \times 10^{10}$	15 $\mu$ l/TD	4
④ KD-2 (970611)	—	estim $4-5 \times 10^{10}$	20 $\mu$ l/TD	4
⑤ KD-3 970617 <sup>~15me</sup> 32g sup	—	estimated $2-3 \times 10^{10}$	{ 25 $\mu$ l/TD 40 $\mu$ l/TD	3 1 (Ara-C TD)
		150cm <sup>2</sup> flask		

Added 1/2 ml DME (10% FCS) at 3:30pm.

Added 4X Ara-C<sup>(10  $\mu$ l of stock)</sup> at ~6pm & again at 9Am on (6/20), fixed Ara-C & one set of coverslips at 27h post-infr. (other ss's fixed at 49 hours post-infection → all fixed in Paraformaldehyde followed by MeOH/DAPI, then a 2 min MeOH rinse & rehydrated in PBS; stained on (6/23) [after checked out Baolings antibodies]

EXHIBIT

41

970623

## Immunofluorescence Staining

all fixed in PF/meth w DAPI

Set #1

(M73(1:2)

121531 (1:1000 find)

600  $\mu$ l

- 1 mock (Ara-C) slight bckgrd (all  $\alpha$  Ab)
- 2 dl 309 (Ara-C) 400% inf'd; some Golgi
- 3 dl 309 (27h) Golgi + speckled around nuclei
- 4 KD-1 (Ara-C) 80-90% inf'd; some Golgi
- 5 KD-1 (27h) much more than Ad5; very vesicular
- 6  $\downarrow$  (49h) all cells bright (all vesicular)
- 7 KD-2 (Ara-C) 70-80% inf'd; prob. some Golgi
- 8  $\downarrow$  (27h) more like Ad5, but some vesicular
- 9  $\downarrow$  (49h) more diffuse; some cells quite bright; some Golgi
- 10 KD-3 (Ara-C) 50-60% inf'd; pretty similar to Ad5 (AC)
- 11  $\downarrow$  (27h) not too much staining
- 12  $\downarrow$  (49h) some cells brighter (again rather "diffuse")

Set #2 ( $\alpha$ DBP /  $\alpha$ Fib each)250  $\mu$ l

- 13 mock (49h) slight backgrd (esp. FITC)
- 14 309 (27h) all nuclei stain for both
- 15 KD-1 (49h) strong staining for both
- 16 KD-2  $\downarrow$  more stained for DBP
- 17 KD-3  $\downarrow$  fewer cells stained for Fib

Volume of 1: 2<sup>nd</sup> : 309 (10  $\mu$ l), KD-1 (15  $\mu$ l), KD-2 (20  $\mu$ l), KD-3 (40  $\mu$ l Ara-C, or 25  $\mu$ l)

1<sup>st</sup> Ab(s): 50  $\mu$ l for 60 minutes;

PBS rinses: 70 min, 12 min;

2<sup>nd</sup> Ab(s): Goat  $\alpha$  rabbit FITC - & Goat  $\alpha$  mouse FITC (not preadsorbed)  
1:50 dilution of each  
50  $\mu$ l for 34 minutes;

PBS rinses: 6 1/2 min, 5 min;  
add H<sub>2</sub>O dip  
mounted in Elvanol w p-phenylenediamine



# 970(5/20) Plaque Assays (New Counts)

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
① dl751	$.5 \times 10^{-8}$	A	76	74	Tmtc	→			
		B	65	66	Tmtc	→			
	$.5 \times 10^{-9}$	A	10	12	10	9	0	0	0
		B	5	6	bad.	5	0	0	Tmtc
		C	4	6	10	10	2	0	2
	$.5 \times 10^{-10}$	A	2	0	2	3	0	0	0
		B	0	0	0	0	0	0	0
		C	1	0	1	0	0	0	0

② dl707 (4/23/97)	$.5 \times 10^{-8}$	A	10	37	13	30	31	10	Tmtc
		B	6	37	15	17	26	11	Tmtc
	$.5 \times 10^{-9}$	A	3	3	2	2	2	5	0
		B	0	6	0	2	5	3 <sup>(bad)</sup>	bad
		C	0	2	0	0	12	9	2
	$.5 \times 10^{-10}$	A	0	0	0	0	1	0	0
		B	0	0	0	0	0	0	0
		C	0	0	3	0	1	0	0

③ dl707 (3/15/97)	$.5 \times 10^{-8}$	A	16	41	14	68	Tmtc	19	→
		B	44	51	15	47	Tmtc	28	→
	$.5 \times 10^{-9}$	A	2	2	2	8	13	3	0
		B	1	8	5	2	8	5	2
		C	0	2	2	9	14	6	2
	$.5 \times 10^{-10}$	A	0	0	0	0	1	0	0
		B	0	0	0	0	0	1	0
		C	0	0	0	0	0	3	0

EXHIBIT

#4

# 970520 Plaque Assays

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
④ PME	$.5 \times 10^{-8}$	<del>A</del>	42	80					
		A	<del>37</del>	<del>102</del>	74	Tmtc			
	$.5 \times 10^{-9}$	B	37	102	78	Tmtc			
		A	1	8	16	12	4	0	Tmtc
		B	7	17	11	14	1	0	Tmtc
	$.5 \times 10^{-10}$	C	7	15	20	7	11	0	Tmtc
		A	0	0	4	1	2	1	2
		B	0	0	3	0	1	0	0
		C	0	2	2	2	0	2	0

⑤ KD	$.5 \times 10^{-8}$	A	Tmtc						
		B	Tmtc						
	$.5 \times 10^{-9}$	A	20	8	8	3	5	0	0
		B	37	7	2	1	2	0	0
		C	24	11	11	1	0	0	0
	$.5 \times 10^{-10}$	A	2	0	1	1	1	2	0
		B	0	1	0	0	0	1	0
		C	5	0	0	2	1	2	0

EXHIBIT

15



# 970(5/20) Plaque Assays

Cumulative Counts

Virus	Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
① dl751	$.5 \times 10^{-8}$	A	76	150	TMTc	→			
		B	65	131	TMTc	→			
	$.5 \times 10^{-9}$	A	10	22	32	41	41	41	41
		B	5	11	11	16	16	16	16
		C	4	10	20	30	32	32	34
	$.5 \times 10^{-10}$	A	2	2	4	7	7	7	7
		B	0	0	0	0	0	0	0
		C	1	1	2	2	2	2	2
			22	46	69	96	98	98	100
	$.5 \times 10^{-8}$	A	10	47	60	90	121	131	TMTc
		B	6	43	58	75	101	112	TMTc
	$.5 \times 10^{-9}$	A	3	6	9	11	13	18	18
		B	0	6	6	9	14	17	17
		C	0	2	2	2	14	23	25
	$.5 \times 10^{-10}$	A	0	0	0	0	1	1	1
		B	0	0	0	0	0	0	0
		C	0	0	3	3	4	4	4
			3	14	20	25	46	63	65
③ dl707 (3/15/97)	$.5 \times 10^{-8}$	A	16	57	71	139	TMTc	→	
		B	44	95	110	157	TMTc	→	
	$.5 \times 10^{-9}$	A	2	4	6	14	27	30	30
		B	1	9	14	16	24	29	31
		C	0	2	4	13	27	33	35
	$.5 \times 10^{-10}$	A	0	0	0	0	1	1	1
		B	0	0	0	0	0	1	1
		C	0	0	0	0	0	3	3
			3	15	24	43	79	97	101

EXHIBIT

#6

# 970520 Plaque Assays

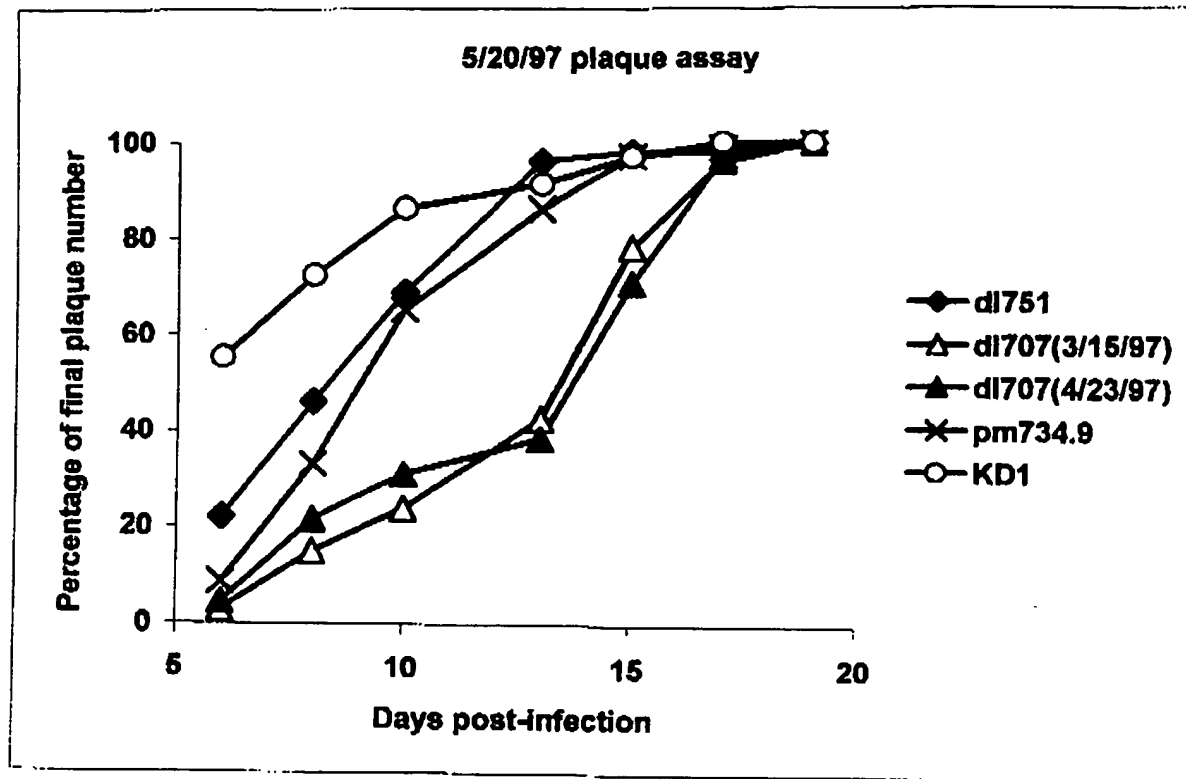
Virus Dilution	Dish	5/26	5/28	5/30	6/2	6/4	6/6	6/8
④ PmE $.5 \times 10^{-8}$	A	42	122	196	TmTC			
	B	37	139	217	TmTC			
	A	1	9	25	37	41	41	TmTC
		7	24	35	49	50	50	TmTC
		7	22	42	49	60	60	TmTC
	B	0	0	4	5	7	8	10
		0	0	3	3	4	4	4
		0	2	4	6	6	8	8
		15	57	113	149	168	171	173
	A	TmTC						
		TmTC						
	A	20	28	36	39	44	44	44
		37	44	46	47	49	49	49
		24	35	46	47	47	47	47
⑤ KD $.5 \times 10^{-9}$	A	2	2	3	4	5	7	7
		0	1	1	1	1	2	2
		5	5	5	7	8	10	10
		88	115	137	145	154	159	159

EXHIBIT

#7

970520 plaque assay\*

	6	8	10	13	15	17	19
dl751	22	46	69	96	98	98	100
dl707(3/15/97)	3	14.9	23.8	42.6	78.2	98	100
dl707(4/23/97)	4.6	21.5	30.8	38.5	70.8	98.9	100
pm734.9	8.7	32.9	65.3	86.1	97.1	98.8	100
KD1	55.3	72.3	88.2	91.2	96.9	100	100



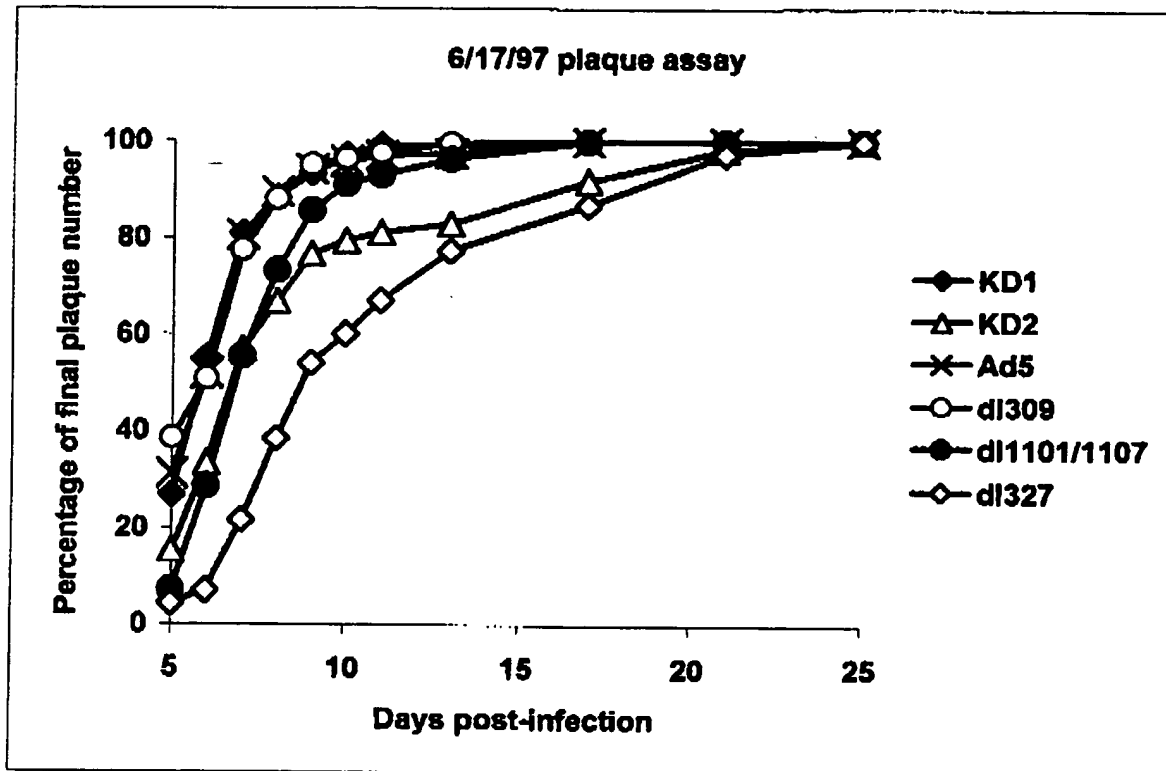
\*numbers at the top of the page are the data points represented in the graph

EXHIBIT

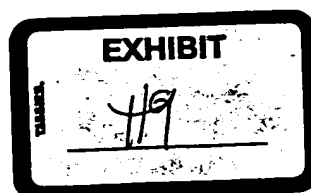
48

970520 plaque assay\*

	5	6	7	8	9	10	11	13	17	21	25
KD1	27	55.2	80.9	88.4	93.4	97.1	99.2	99.2	100	100	100
KD2	15.3	33.3	56.8	66.7	76.6	79.3	81.1	82.9	91.9	98.2	100
Ad5	31.2	51.9	80.6	89.5	94.1	95.8	97	97.5	100	100	100
dl309	38.5	50.8	77.5	88.1	95.1	96.3	97.5	99.6	100	100	100
dl1101/1107	7.1	28.6	55.4	73.2	85.7	91.1	92.9	96.4	100	100	100
dl327	4.3	7	21.7	38.3	53.9	60	67	77.4	87	97.4	100



\*numbers at the top of the page are the data points represented in the graph



(7/26) Blue

(5/28) Green

H6

970526

(5/20) Plaque Assays

<u>Urine</u>	<u>Dilution</u>	<u>A</u>	<u>B</u>	<u>C</u>	<u>Comments</u>
① dl 751	$0.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	76+74 10+12 2+0	65+66 5+6 0 <sup>0</sup>	<del>4+6</del> 1+0	quite large + very distinct
② dl 707 (4/23)	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	10+37 3+3 0 <sup>0</sup>	6+37 0+6 0 <sup>0</sup>	<del>0+2</del> 0 <sup>0</sup>	small, flat + very indistinct
③ dl 707 (3/15)	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	16+41 2+2 0 <sup>0</sup>	44+51 1+8 0 <sup>0</sup>	<del>0+2</del> 0 <sup>0</sup>	flat + <u>indistinct</u>
④ PME	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	46+80 1+8 0 <sup>0</sup>	37+102 7+17 0 <sup>0</sup>	<del>7+15</del> 0+2	fairly large + quite distinct
⑤ KP	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	TNTC 20+8 2+0	TNTC 37+7 0+1	<del>24+11</del> 5+0	small to large all distinct; "cleared" center in many plaques (all cells lysed?)
⑥ mock	—	0	0	<del>—</del>	

AT Counts + descriptions of Shari O'Briens

(5/20) Plaque Assay

Comments most likely done on  
15/1/1

EXHIBIT

H10

# Analysing the infectivity of the viruses 1101/1107 and d1309 on Growth arrested and growing cells.

cell line = HEL 299 human Lung embryonic.

Grown as in ATCC literature

T. 75 flask 1 from Karl,

split to 1:30 = 35 mm dish.  
1:60 = 35 mm dish. (how many)  
(for growing conditions)

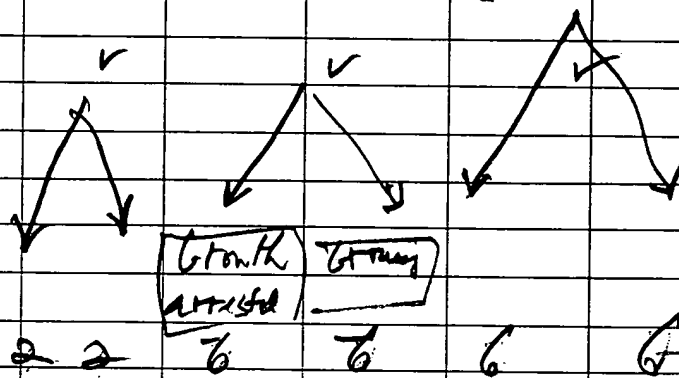
35 mm dish.

uninfected

1101/1107

d1309

1) 35mm + cover glass



(28)

2) 35mm + coverslip =

how many days? 6 days

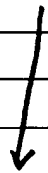
↓  
titer each point 1-6 days or

EXHIBIT

I

# Analysis of the dl 1101/1107, dl309 viruses infectivity on Growth arrested and growing HEI 293 cells:

HEI 293 one T-75



35 mm dish

1:30 split for growth arrested

1:60 for growing cells

Growing growth arrested

1. Uninfected - mock

2. 1101/1107 infected

3. dl 309 infected

1 (1)

1+2

2

2 (1)

2

2 (1)

(12)



look for CPE - days?

4. On cover slip

1, 2, 3

days

for Ad fiber IF.

(1+6)

(10)

(22)

virus location

dl 309 = pfu ✓

dl 1101/1107 = pfu ✓

EXHIBIT

12

HEL 299

↓ 7.75

35 mm dishes

1:30,

7:60

↓

MEM; 0.1mM Non essential a.a.s; 1mM Sodium -  
pyruvate + 0.1% lactalbumin hydrolysate.  
+ 10% FBS

↓

1:60 Split, count the cells + infect E

dl 110/11071, dl 309, 100 moi

↓

1:30 Split, allow to become confluent, then growth  
arrest by low serum, (3 days) then infect E dl 1101/dl  
dl 309 0.2% 1107

↓

EXHIBIT

43



viral infection dl 1101/1107

- count one growing MEL 299 cells.  
- 100 pfu -

- Make 200 ml EMEM + Na<sub>2</sub>Aspartate, NEAA + Lactalbumin hydrolyzate  
100x 100x 100x

$$\frac{116}{31} = 3.74 \quad 347 \times 2 = 294 \quad \boxed{294,000 / \text{ml}} \checkmark$$

dl 309 - 950111 - pfu  $2.15 \times 10^{11}$  Counted 5-7  
dl 1101/1107 - from Karl - pfu  $1.95 \times 10^{11}$

from 35 mm - MEL 299 growing cells - counted - 294,000 / ml

$$\begin{aligned} 100 \text{ pfu dl 309} &= \frac{100 \times 294,000}{2.15 \times 10^{11}} \\ &= \frac{294}{2.15 \times 10^6} = \frac{2.94}{2.15 \times 10^4} = 0.00013 \\ &= \text{virus is too concentrated. Dilute to } 10^9 \\ 10 \text{ ml dilute to } 1 \text{ ml in medium (no serum) gives} &= 2.15 \times 10^9 \end{aligned}$$

EXHIBIT

24

$$= \frac{100 \times 294,000}{2.15 \times 10^4} = \frac{294}{2.15 \times 10^4} = 13,440$$

dl 1101 / dl 1107 100 pfu =

~~100~~ x 294,000

294

dilute the virus to  $10^4$   
in 10  $\mu$ l to 1 ml

$1.95 \times 10^4$

$1.95 \times 10^4$

= 15  $\mu$ l

12/16/96

Growing cells

2 x 35 mm dish dl 309, 100 pfu

2 x 35 mm dish dl 1101/1107, 100 pfu

6 x 35 mm on coverslip dl 1101/1107 100 pfu

EXHIBIT

75

5

21/10/1977

Cell	Wants	=	341,000
------	-------	---	---------

$$\Delta 308 = 100 \text{ pfn} = \frac{341 \times 10^2 \times 100}{2.15 \times 10^4} = 15.8 \text{ ml}$$

$$d_1 \text{ 1001 } = 100 \text{ pfg} = \frac{341 \times 10^3 \times 100}{1.95 \times 10^9} = 17.4 \mu\text{m}$$

Vin 10 ml to 1ml  $\rightarrow 10^9$

↓  
1hr 37 i / 1hr

Medien + 0.2 g Des.

**EXHIBIT**

26

# Fixing the HEL 299 cells + Methanol + DAPI (1mg/ml)

(1pt to 1ml methanol)

1. Wash the cells 2x PBS w/o
2. Fix + Methanol (-20°C) + DAPI ~ 8 min (-20°C)
3. Wash in Methanol w/o DAPI
4. Wash in PBS, store at 4°C

✓	1 day	12/11		
✓	2 day	12/18		
✓	3 day	12/19/96	→	2 d1309 started showing CPE
✓	4 day	12/20	NO CPE	
	5 "	12/21	NO CPE	- d1309 complete CPE
	6 "			
	7 day	12/23	NO CPE	) all d1309 cells infected rounded + came off
	8 day	12/24	NO CPE	
	9 "	12/25		
→	10 day	12/26	LPE?!	cells looks different as d1101/1107
F	11	12/27		
S	12	12/28		
Sm	13	12/29	— CPE? starts	on 12th day d1101/1107
✓ M	14	12/30	— CPE?	spreads - d1101/1107
	15	12/31	"	"
	16	1/1		
✓	17	2/1	→	Take photographs -
	18	3/1	→	
	19	4/1	→	
	20	5/1	→	almost all the cells died - d1101/1107
	25	12/1	→	Stain - dish, 2 crystal violet
				2 d1101/1107
				2 - d1309



# Growth arrested HEL 299 cells:

Date	Days	
8	15	NO visible CPE in all 1101 / 1107: some cells are floating. In normal HEL 299 cells some cells are floating but less.
9	16	same as above.
10	17	
11	18	same
12	19	cells looking different than norm
13	20	<del>HE1299</del> BUT, d1309 CPE looks different
14	21	
15	22	
16	23	" CPE?!
17	24	" CPE?!
18		

EXHIBIT

18

HEL299 cells

Kodak

17 days

7

Tmax 100  
black white printGrowing HEL 299

1.	MOCK	N
2	"	+1
3	"	-1
4	"	-1
5	"	N
6	"	+1
7	d1110/1107	N
8	"	+1
9	"	-1
10	"	N
11	"	+1
12	"	-1
13	"	N
14	"	+1
15	"	-1
16	"	N
17	"	+1
18	"	-1
19	"	N
20	"	+1
21	"	-1
22	"	N
23	"	+1
24	"	-1
25	"	N
26	"	+1
27	"	-1
28	"	N
29	"	+1
30	"	-1
31		
32	"	N
33	"	+1
34	"	-1
35	MOCK	N
36	MOCK	+1

TMAX

EXHIBIT

29

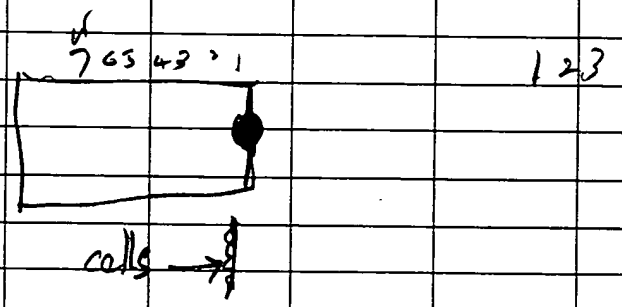
IF: HEL 299 : Growing:

1. ab - Filter, → 1:400  
 DBP → 1:400  
 30'  
 2. Goat anti mouse Rhodamine 1:50 (not preabsorbed)  
 Goat anti Rabbit FITC 1:25

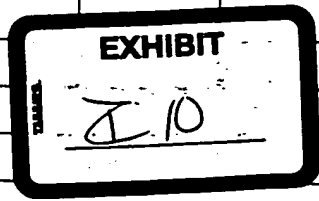
1 = 1<sup>st</sup> layer  
 2 = 2<sup>nd</sup>  
 3 = 3<sup>rd</sup>  
 4 = 4<sup>th</sup>  
 5 = 14<sup>th</sup>  
 6 = 17<sup>th</sup>  
 7 = CTRL  
 1101/1107  
 - ? < p  
 - little broken

FITC = DBP  
 FITC = FITC  
 2 DBP p 67  
 66420  
 850625  
 402-5  
 ascites fluid

1. ab = Ad Fiber dil 1:400  
 = DBP dil 1:400  
 in 1 tube  
 35 µl / dish → 37°C / 30 min  
 wash: 2 x PBS w/o, 10 min x 3 times



2. Goat α mouse Rhodamine 1:50  
 Goat α Rabbit FITC 1:25  
 37°C / 15 min  
 960125  
 8 → 400  
 16 → 400  
 16  
 400



LITHOGRAPHED IN U.S.A. ADDISON-WESLEY PUBLISHING COMPANY, INC., READING, MA 01860 1-800-447-2226 A-10 Dataform 87927 692 104G

# Growth Assay

Growth arrested cells.

HEL 299

T-75 → split 15 x 35 mm dish.

\* dishes same like growing

↓  
add EMEM + 0.2% FCS

↓  
3 days

↓  
infect  $\bar{e}$  dl 1101 / 1102  
pm 975

the control dl 309.

total dish # 27

EXHIBIT

CP 11



HEL 299 T-75

# Growth Assay

Growing:

↓ split to 35 x 35 mm dish

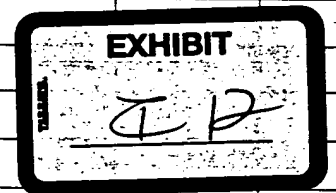
① 1 + 10 dish days (1, 4, 6, 8, 10, 12, 14, 16, 18, 20)  
dl 1101/1107

② 1 + 10 dish Same days - pm 975

↓ infect 100 pfu, freeze the dishes as days shown.

③ 4 dishes infect & dl 309 for true infection  
Freeze 1, 4, 6 days.

total dishes 27



1/14/98

Infection	dl 1101/1107	pfu $1.95 \times 10^{11}$
	dl 309	$2.15 \times 10^{11}$
	pm 975	$4.06 \times 10^{10}$

Growing HEL 299 cells counted: 212,000/ml

① 100 pfu

For dl 1101/1107 =  $\frac{100 \times 212,000}{1.95 \times 10^{10}} = \frac{2.12}{1.95 \times 10^2} = 10.8 \mu$   
 Dilute the virus to  $10^9$   
 10 ml to 1ml medium No serum

② For pm 975 =  $\frac{100 \times 2.12 \times 10^5}{4.06 \times 10^9} = \frac{2.12}{4.06 \times 10^2} = 5.2 \mu$   
 dilute the virus to  $10^9$   
 50 ml to 500 ml serum free media

# Growth Curve

## Growing cells: Summary

③ the CTRL

virus dil 300  
dilute the virus to 10<sup>9</sup>  
10 µl to each serum free  
medium

$$= 10 \times 2.12 \times 10^8$$

$$\frac{2.12 \times 10^8}{2.15 \times 10^8} = 2$$

$$\frac{2.12}{2.15 \times 10^2} = 9.8 \times 10^{-3}$$

1. wash the cells to PBS 4x
2. infect in serum free medium 37°C / 1 hr
3. After 1 hr, remove the medium, wash to 1x medium
4. Add MEM + 10% FCS.

Freeze the cells after

1-17-97	1 day	G1	1/15/97 ✓
1-19/97	3 day	G2	dil 300, PM975 shows CPE NO CPE in dil 1101/1107
	5	G3	N 60% CPE in pm 975 NO CPE in dil 1101
	6	G4	pm 975 ✓ NO CPE dil 1101
	7	G5	pm 975 ~ 100% ✓ G3 dil 1101/1107 ~ CPE? - NO
10	10		PM975 dil 1101/1107 (NO CPE 4x) dil 300 (Photos)
	15		dil 1101/1101 (morphology change CPE?)
	24		dil 1101/ G5

EXHIBIT

713

# Growing II

infected 1/31/97

		100	500	100	500	1	100	500
		d1309	PM 975	d1101	d107			
Day 1		NO CPE "	NO CPE "	NO CPE "				
Day 2		Few cells rounds	Few cells rounds	Few cells rounds		NO CPE "		
Day 3		10% CPE - 20% HT	20% 50% CPE	NO CPE				
Day 6		100% CPE in NO CPE in	PM 975; d1309 d1101/1107					
Day 14		—	—	500 pfu - CPE starts.				
Day 17		—	—	100 pfu - CPE starts 500 pfu - CPE +				

EXHIBIT

7-14

# Growth Arrested

MEL 288

122/57

G1

Days

3

pm 975 - -70°C

5

pm 975, dl 1101/1107, dl 309

(photos)

7

pm 975 -70°C (photos)

10

pm 975; dl 1101/1107 (NO LPE)

(GAT)

(GAT)

14

pm 975

(GAT)

15

- pm 975 NO GAT

501 cells from control & pm 975. dl 1101/1101  
and control org. NO contamination.  
Maybe due to not changing mesh?

19

pm 975 (GAT)

EXHIBIT

15

Growing cells  
on cover slips  
for IF & CPE

HEL 299 cells

counted 1 dish = 188,000 cells = (1:35 dil)

① virus pm 925 pfu  $4.06 \times 10^{10}$

for 100 pfu =  $\frac{100 \times 1.88 \times 10^4}{4.06 \times 10^{10} \times 2} = \frac{1.88}{4.06 \times 10^2}$   
dilute the virus to  $10^9$  titer.  
50  $\mu$ l to 500  $\mu$ l serum free media = 4.6  $\mu$ l

② virus dl 101/1107

=  $\frac{100 \times 1.88 \times 10^4}{1.95 \times 10^9 \times 2} = \frac{1.88}{1.95 \times 10^2}$   
dilute the virus to  $10^9$   
10  $\mu$ l to 1ml medium.  
= 9.6  $\mu$ l

③ +ve control dl 309

=  $\frac{100 \times 1.88 \times 10^4}{2.15 \times 10^9 \times 2} = \frac{1.88}{2.15 \times 10^2}$   
dilute the virus to  $10^9$   
10  $\mu$ l to 1ml medium  
= 8.7  $\mu$ l  
10  $\mu$ l

EXHIBIT

2/16

# Growth arrested HEL 299<sup>P12</sup> cells P<sup>II</sup>

24 days

0	HEL 299 (Mock)	= 0
1	"	+1
2	"	-1
3	"	0
4	"	+1
5	"	-1
6	HEL 299 + dl 1101/1107	= 0
7	"	+1
8	"	-1
9	↑ ↓	
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
Growing cells 3 <sup>rd</sup> days		
21	HEL 299 Mock	= 0
22	"	+1
23	"	-1
24	+ PM 125	=
25	"	
26	"	
27	dl 1101/1107	
28	"	
29	"	
30	dl 309	
31	"	
32	"	
33	PM 935	
34	↓	
35		
36		
37		

EXHIBIT

17

EXHIBIT

17

Growth arrested HEL 299 cells  
 injected E d1101/1107 24<sup>th</sup> day

1	HEL 299 (mole)	= 0	TMY 400 B+W
2	"	+1	
3	"	-1	
4	"	0	
5	"	+1	
6	"	-1	
7	HEL 299 + d1101/1107	0	print reads 0=1
8	"	+1	
9	"	-1	
10	"	0	
11	"	+1	
12	"	-1	
13	"	0	
14	"	+1	
15	"	-1	
16	"	0	
17	"	+1	Same
18	"	-1	
19	"	0	
20	"	+1	
21	"	-1	

growing 21, 22, 23 mole

21	HEL 299 MOLE	= 0	
22	"	+1	
23	"	-1	
24	+ PM 975	0	
25	"	+1	
26	"	-1	
27	d1101	0	
28	"	+1	
29	"	-1	
30	d1309	0	
31	"	+1	
32	"	-1	
33	PM 975	0 0	
34	"	+1	
35	"	-1	
36	"		
37	"		

Growing cells 3<sup>rd</sup> day

Same

EXHIBIT  
 IB

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 ADL-JUN-WESLEY PUBLISHING COMPANY, INC., READING MA 1-800-447-2226  
 A-W Dataform © 87927002 104G

# Growth arrested HEL 299 cells

## - Growth Curve:-

Count the cells: 347,000 cells

① 100 pfu =  
for dilution 10<sup>4</sup> =  $\frac{100 \times 347,000}{1.95 \times 10^4} = \frac{347}{1.95 \times 10^4} = 17 \text{ ul}$

dilute the virus to 10<sup>4</sup>  
10 ml to 1 ml

② 100 pfu  
for pm 975 =  $\frac{100 \times 347,000}{4.01 \times 10^4} = \frac{347}{4.01 \times 10^4} = 8.5 \text{ ul}$

dilute the virus to 10<sup>4</sup>  
50 ml to 500 ml

③ the count is 309 =  $\frac{100 \times 347,000}{2.15 \times 10^4} = \frac{347}{2.15 \times 10^4} = 16 \text{ ul}$

dil to 10<sup>4</sup>

10 ml to 1 ml

↓  
work  
infant  
EMEM 0.29 FCS

EXHIBIT

19



III

photographs:

Tmax 170 B/w.

Growth arrested HEL299, 3 days

C

1820

A-W Dataform © 87977 692

ADDISON-WESLEY PUBLISHING COMPANY, INC., READING, MA 01860-447-2226

LITHOGRAPHED IN U.S.A.

1	HEL299 - MULK	= 0
2	"	+1
3	"	-1
4	"	0
5	"	+1
6	"	-1
7	+ pm 975	0
8	"	+1
9	"	-1
10	"	0
11	"	+1
12	"	-1
13	"	0
14	"	+1
15	"	-1
16	"	0
17	"	+1
18	"	-1

pm 975, 3<sup>rd</sup> day, 6A

nucleus appears darker than control

↓ KRT1-

EXHIBIT

420

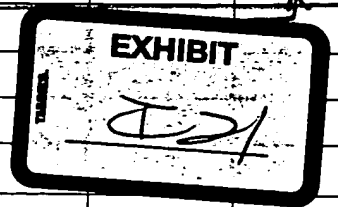
PTV  
in photos

Growing 4<sup>th</sup> day

Tmax 100

B/W. Prints

1	HEL 299 (Mock)	2 NCO	
2	"	H	
3	"	-1	
4	"	0	
5	"	+1	
6	"	-1	
7	dl 309	0	
8	"	+	
9	"	-1	CPE ✓
10	"	0	
11	"	+1	
12	"	-1	
13	pm 975	0	
14	"	+1	
15	"	-1	CPE ✓
16	"	0	
17	"	+	
18	"	-1	
19	dl 1101	0	
20	"	+1	Δ ?
21	"	-1	
22	"	0	0
23	"	+1	
24	"	-1	
Growth arrested 5 <sup>th</sup> day			
25	HEL 299 Mock	0	
26	"	+1	
27	"	-1	
28	pm 975	0	
29	"	+1	
30	"	-1	CPE - Not yet
31	"	0	NO CPE sign
32	"	+1	
33	"	-1	
34	dl 309	0	
35	"	+	Shows - starting CPE
36	"	-1	
37	"	0 less light	



8/

# Photographs

Carl took 1-18 photos

	Growth	Arrested	HEL 289	9th day
19	HEL LA	0		
20	"	+1		
21	"	-1		
22	PM 925	0		
23	"	+1		
24	"	-1		
25	"	0		
26	"	+		
27	"	-1		
28	AL 1107	0		
29	"	+1		
30	"	-1		
31		0		
32		+		
33		-1		
34	AL 309	0		
35	"	+		
36	"	-1		
37	"	0		
38	"	+1		

NO CPE sign

NO CPE sign

Shows CPE

EXHIBIT

122

P'6

photos:

Tmax 100 Black & white

Growth arrested HEL 299 - 10<sup>th</sup> day.

1 HEL 299, GA, mock - 0

2 " " +1

3 " " -1

4 PM975 - 0

5 " " +1

6 " " -1

7 " " 0

8 " " +1

9 " " -1

10 dl 1101 / 1107 - 0

11 " " +1

12 " " -1

13 " " 0

14 " " +1

15 " " -1

16 dl 309 - 0

17 " " +1

18 " " -1

19 " " 0

20 " " +1

21 " " -1

NO CPE?

NO CPE.

shows CPE - growing

Growing cells → 15 days

22 HEL 299 - mock ± 0

23 " " +1

24 " " -1

25 PM975 - 0

26 " " +1

27 " " -1

28 dl 309 - 0

29 " " +1

30 " " -1

31 dl 1101 / 1107 0

32 " " +1

33 " " +1

34 dl 1101 / 1107 - 0

All the cells rounded & most of them floating (dead cells)

All the cells rounded & floating (dead)

Cells are larger, fewer, some cells have larger nuclei - CPE?

EXHIBIT

223

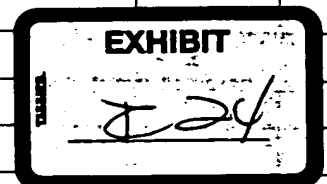
A. W. Wesley Publishing Co., Inc., Reading, MA 01063

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LITHOGRAPHED IN U.S.A.

photographs 3<sup>rd</sup> day:  
GROWING HEL 299 P<sup>17</sup>

1	HEL 299 G	2	0		
2	"		+1		
3	"		-1		
4	dl 309	100 pfn	0		
5	"		+1	100 pfn	~ 10% UG
6	"		-1		
7	dl 309	500 pfn	0		
8	"		+1	500 pfn	~ 30%
9	"		-1		
10	pm 175	100 pfn	0		
11	"		+1		
12	"		-1		
13	"		0	100 pfn	~ 20%
14	"		+1		
15	"		-1		
16	pm 175	500 pfn	0		
17	"		+1		
18	"		-1	500 pfn	~ 10%
19	"		0		
20	"		+1		
21	"		-1		
22	dl 1101	100 pfn	0		
23	"		+1		
24	"		-1	100 pfn	NO UG
25	"		0		
26	"		+		
27	"		-1		
28	dl 1101	500 pfn	0		
29	"		+1		
30	"		-1	500 pfn	NO UG
31	"		0		
32	"		+1		
33	"		-1		
34	HEL 299 CONTROL		0		
35	"		+1		
36	"		-1		



p 8

photographsGrowing 24 hr day-

1	HEL 299.6	=	0	
2	"		+1	
3	"		-1	
4	"		0	
5	"		+1	
6	"		-1	
7	dl 1101/1107		0	
8	"		+1	
9	"		-1	
10	"		0	skips (0)
11	"		+1	
12	"		-1	
13	"		0	
14	"		+	
15	"		-	
16	GA HEL 299	19 days	0	
17	HEL 299 GA		+1	
18	"		-1	
19	"		0	cells are deging
20	"		+1	
21	"		-1	
22	pr 925		0	
23	"		+1	
24	"		-1	
25	"		0	
26	"		+1	
27	"		-1	
28	dl 1101/		0	
29	"		+1	
30	"		-1	
31	"		0	
32	"		+1	
33	"		-	
34	HEL 299 C		0	
35	"		+1	
36	"		-1	

EXHIBIT

E-25

Expt 3

Growth arrested 412-299 cells p<sup>17</sup>  
- Growth curve:-

Count the cells: 347,000 cells

① 100 pfr =  
for d1101/107  $= \frac{100 \times 347,000}{1.95 \times 10^4} = \frac{347}{1.95 \times 10^4} = 17.2$

dilute the virus to 10<sup>9</sup>  
10 ml to 1 ml

500 pfr = 85.0

② 100 pfr  
for pm 975  $= \frac{100 \times 3,47 \times 10^4}{4.06 \times 10^2} = \frac{3.47}{4.06 \times 10^2} = 8.5 \text{ ml}$

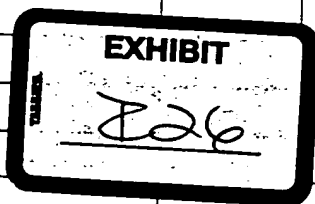
dilute the virus to 10<sup>9</sup>  
50 ml to 500 ml

500 pfr: 42.5 ml

③ the CONTR d1309  $= \frac{100 \times 3.47 \times 10^4}{2.15 \times 10^2} = \frac{3.47}{2.15 \times 10^2} = 16 \text{ ml}$

10 ml to 1 ml

500 pfr = 80 ml



↓

wash  
infant

EMEM 9-29 FCS  
NO CPE

Days 1, 2 =

3/10/97  
F

Days 3:

d1101, pm 975, d1309 NO CPE / 100 pfr  
at 500 pfr, 2 pm 975 & d1309 shows  
beginning of CPE

Day 4 = NO CPE 1101/1107 (100 d 500 pfr)

Day 5 = NO CPE PM 975 / 100 pfr

= CPE begins pm 975 500 pfr only

Day 6 = NO CPE PM 975 100 pfr

2/13/97

Day 7 =

NO CPE d1101/100 & 500 pfr

NO CPE d1309 100 pfr. CPE in d1309 500 pfr

Growth Curve: Frozen: days:

KEI-299

Growing: pm975, dl1101  
1, 3, 5, 6, 7, 1, 3, 5, 6, 7, 10, 15, 19  
GA: 3, 5, 7, 10, 14 5, 7, 10 19

pm975 GA: GA  
✓GA 3 = 14th day  
✓GA 4 = 10th  
GA 2 = 5th  
✓GA 7 = 19th  
GA 1 = 3th  
Growing  
✓26 = 3th day 67  
✓36 = 5th " 67  
✓46 = 6th " 64  
✓56 = 7th "

dl 1101/1107 = GA  
✓GA 7 = 19th day  
✓GA 9 = 10th "  
✓GA 2 = 5th "  
✓GA 23 = 23th "  
Growing  
G 3 = 5th day  
G 4 = 15th "  
G 24 = 24th day

2/18/97

pm975: GA 10, 14, 19 day  
Growing = 3, 5, 6, 17 day  
307?

EXHIBIT

227



# Growth Curve - Plaque assay:

293 cells:-

Growing : pm 975 : days 1, 3, 5, 6, 7

GA : pm 975 = 3, 5, 7, 10

# 9

Growing : dl1101 = 1, 5, 7, 10, 15

GA = 7, 10

# 7

dl309 =

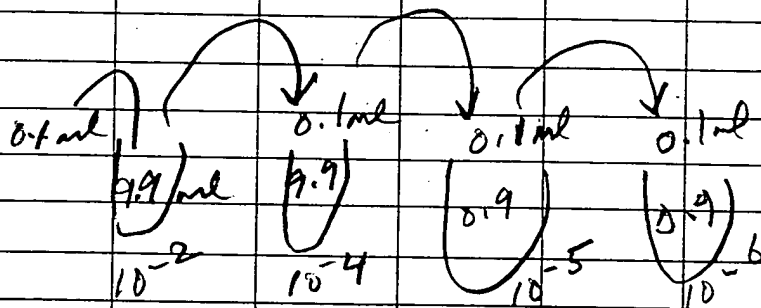
infections = 0.5 ml wt  
0.1 ml  $10^{-2}$   
 $10^{-4}$   
 $10^{-5}$

DO pm 975 first  
# 9 points Growing = 5

GA = 4

= 5x = 25 dish  
= 4x = 20 dish

35



0.5 ml suspension:  
1 ml  $10^{-2}$  to  $10^{-6}$

0.1 ml wt

Strain on 2/24/97

EXHIBIT

228

40 disks:  $\times 5 =$  200 ml

200 ml  
 $\times 2$

(A) ~~50 ml~~ 2x DUEM, 50 ml  
7.5 g Sod.  $\text{HCO}_3$ , 5 ml  
FLS = 2 ml

(B) 1.8 g Agave = 43 ml

dl 1101/1107 Growth Curve:  
plaque assay

dl 1101/1107

GA:

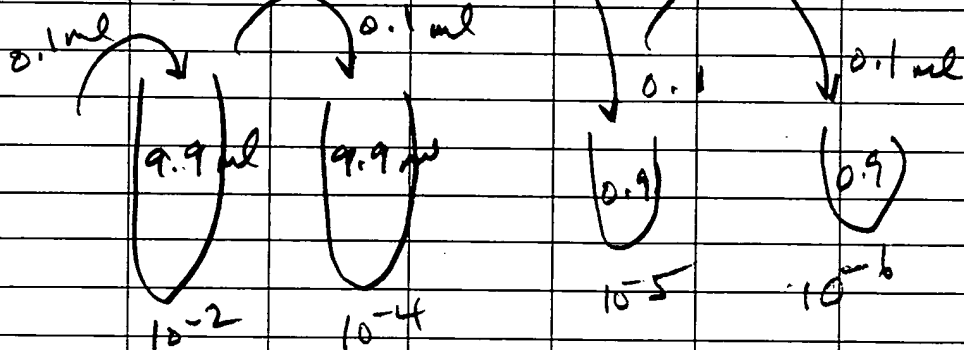
Day  
GA7 = 19=  
GA9 = 10  
GA2 = 5  
GA = 23

Growing

G3 = 5<sup>th</sup>  
G4 = 15<sup>th</sup> day  
G24 = 24<sup>th</sup>

GA5 p0925

Dilutions



↓  
0.5 ml injection:

EXHIBIT

829

GA PM 975 5<sup>th</sup> day.

$$31 \times 10^4 / 8.5 \text{ ne}$$

$$62 \times 10^4 / 1 \text{ ne}$$

$$6.2 \times 10^5 / \text{ne pfa.}$$

→

# Growth Curve

3/4/97

21 1101/1207

100 pfu

-Growing

Small plaques

went one more day:-

$$G3 = 5^{\text{th}} \text{ day} \quad 1.4 \times 10^4$$

$$15^{\text{th}} \text{ day} = 10^{-2} = 406 / 1 \text{ ml} \times 10^2$$

$$= 4 \times 10^4$$

$$24^{\text{th}} \text{ day} = 10^{-4} \quad 66 / 0.5 \text{ ml}$$

$$132 / 1 \text{ ml}$$

$$= 132 \times 10^4$$

$$= 1.3 \times 10^6 / \text{ml}$$

G4

$$5^{\text{th}} \text{ day} = 1.1 \times 10^3 \text{ pfu/ml (No plaques in ud.)}$$

$$10^{\text{th}} \text{ day} = 7 \times 10^2 / 0.5 \text{ ml} = 14 \times 10^2$$

$$= 1.4 \times 10^3$$

$$19^{\text{th}} \text{ day} = 42 \text{ plaques} / 0.5 \text{ ml (small)}$$

$$= 84 / 1 \text{ ml} \times 10^2$$

$$= 8.4 \times 10^3 \text{ pfu/ml}$$

$$23^{\text{rd}} \text{ day} = 28 \times 10^2 / 0.5 \text{ ml}$$

$$= 56 \times 10^2$$

$$= 5.6 \times 10^3 \text{ pfu/ml}$$

EXHIBIT

130

# Growth Curve:

2-25-97

<u>Growing</u>	<u>pm 975:</u>	<u>180 pfu</u>	
<u>3<sup>rd</sup> day</u>	$10^{-6}$	<u>plaque</u> 71	<u>pfu/ml</u> $7.1 \times 10^7$
5 <sup>th</sup> day	$10^{-6}$	13	$1.3 \times 10^7$
6 <sup>th</sup> day	$10^{-6}$	11	$1.1 \times 10^7$
7 <sup>th</sup> day	$10^{-5}$	7	$7 \times 10^6$

<u>GA</u>			<u>pfu/ml</u>
10 <sup>th</sup> day:	$10^{-4}$	12	$1.2 \times 10^5$
14 <sup>th</sup> day	$10^{-4}$	32	$3.2 \times 10^5$
19 <sup>th</sup> day	$10^{-4}$	18	$1.8 \times 10^5$

GA HEL 299 infected  $\bar{c}$  pm 975 did not CPE, but  
it gave viral yield  $\approx 10^4$

5<sup>th</sup> day is plaque assayed to day 2/25/97.  
GA pm 975

EXHIBIT

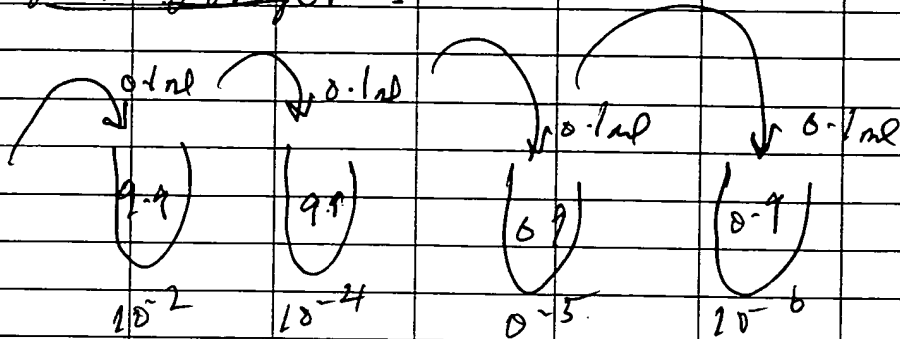
431

# Growth Curve:

3/5/97

pm 975. Growing 1 + 3<sup>rd</sup> log.

~~1102 6-10-97 5<sup>th</sup>~~



EXHIBIT

732

# Growth Curve: plaque assay

3/10/92

Growing:

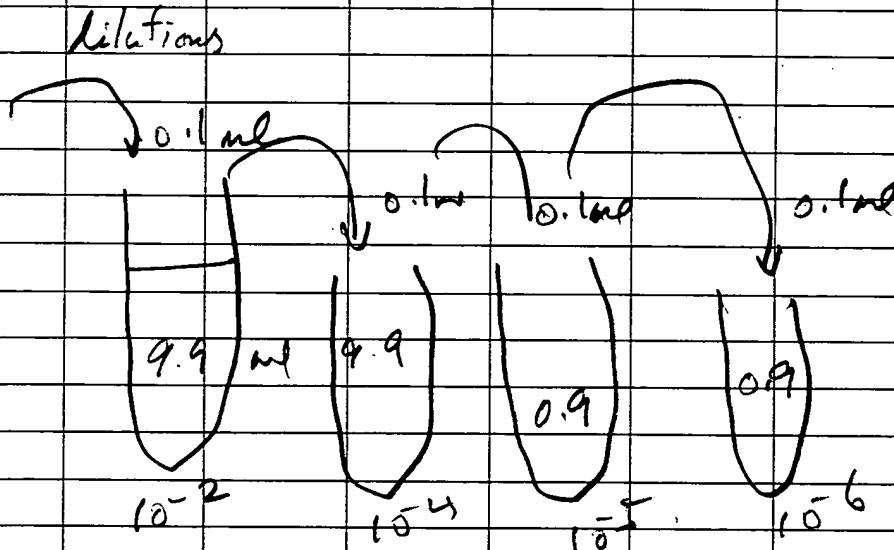
G1 day d1 1101/1107  
G2 (3 day) " /

G1 d1 309  
G5 d1 309

GA = GAZ d1 1101/1107

GAZ, d1 309  
GAS - d1 309  
day

7 viruses ✓



0.5 ml

EXHIBIT

233

St. S. Grand: Amico  
867-6292  
792-10891  
984-2100



Growing:-

Day 1 (G1)

PM 975

$$= 4 \times 10^{-4}$$

$$= \frac{4 \times 10^{-4}}{10^4} = 4 \times 10^{-8}$$

0.5 ml

6<sup>th</sup> day infection

$$= 8 \times 10^4 / \text{ml}$$

Day 3

$$= 19 \times 10^{-5}$$

$$= 1 \times 10^{-6}$$

$$= 1.9 \times 10^6$$

wait 2 more days.

↓ 0.5 ml

$$3.8 \times 10^6 / \text{ml}$$

very small plaques.  
allow two more days to  
form more plaques.

$$31 \times 10^6 \text{ (counted after 2 days)}$$

$$\downarrow$$

$$3.1 \times 10^7 \text{ PFU/ml}$$

EXHIBIT

P 34

# Growth arrested.

3/31/97

500 pfu

HEL 299 = 351,000 cells

1) for dil 1101/97  
dilute virus to  $10^9$   
(10  $\mu$ l to 1ml)

$$= \frac{5}{500} \times 351,000$$

$$= \frac{5 \times 351}{1.95 \times 10^4} = 90 \mu\text{l}$$

2) for dil 309  
dilute the virus to  $10^9$   
(10  $\mu$ l to 1ml)

$$= \frac{5}{500} \times 351,000$$

$$= \frac{5 \times 351}{2.15 \times 10^4} = 81 \mu\text{l}$$

↓ wash  
infect, remove,  
add EMEM + 0.2% FCS.

Freeze) 1 hour 3/31/97 ✓  
1 day  
6  
8  
15

EXHIBIT

75

# CPE Table

Growing (100 phm/cell) AEL 295 - P<sup>17</sup>

d1309 pm975 d1101/107

Day

1

-

-

-

3

+

+

-

5

+++

+++

-

6

++++

++++

-

7

-

10

-

15

+

(cell morphology changes)

24

++

Growth arrested

Day

1

-

-

-

3

±

-

-

5

+

±

-

9

++

-

-

10

+++

-

-

19

+

-

24

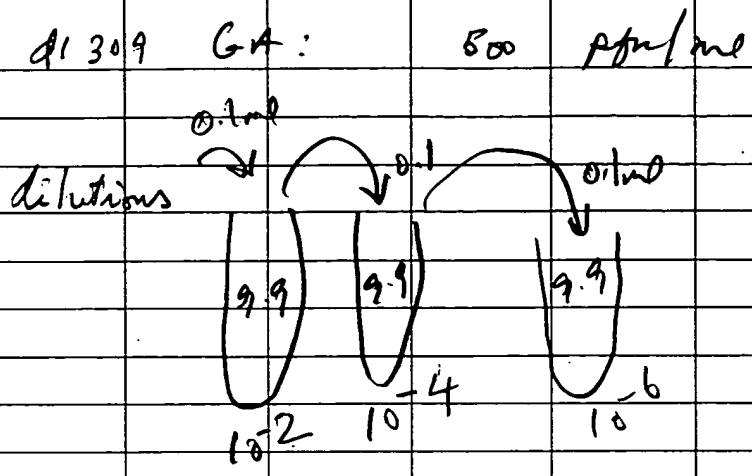
±

-

EXHIBIT

7.30

Titer: 233 cells d1 309 GA MEL 29



3 hr, 1 d, 4, 5, 6, 8 days.  
1 6 & 15

EXHIBIT  
237

HEL 299 cells

5/14/97

5/15/97

5/19/97

500 pfu/cell

Growing

d1309 d1101/07

3 hrs	$9 \times 10^3$	$2 \times 10^4$	-
1 day	$1 \times 10^4$	$4.2 \times 10^4$	-
4 "	$1.6 \times 10^7$	$1.2 \times 10^5$	-
8 " $\rightarrow$	$2.3 \times 10^7$	$1.4 \times 10^5$	-
10 "	$7 \times 10^6$	$2.4 \times 10^5$	$7.1 \times 10^5$
15 "		$2.5 \times 10^5$	$\rightarrow 2.2 \times 10^6$
21 "		$3 \times 10^6$	$\rightarrow 4.6 \times 10^6$

Growth arrested:

3 hrs	$8.9 \times 10^3$	$4 \times 10^4$	
1 day	$6.1 \times 10^4$	$4.4 \times 10^4$	
4 "	$6.2 \times 10^5$	$4 \times 10^4$	
8 " $\rightarrow$	$1.3 \times 10^6$	$5 \times 10^4$	$5.2 \times 10^4$
10 "	-	$1 \times 10^4$	$1.6 \times 10^4$
15 "	$5 \times 10^5$	$1 \times 10^4$	$1.4 \times 10^4$
21 "		$2 \times 10^4$	$2.2 \times 10^4$

$\approx 9 \times 10^3$

#4  
Mohan  
Wolke Bond

EXHIBIT

138

7/12

① harvest  
flasks  
p150

ad 1  
ad 111  
Bailey

② freeze  
② 11-N-⑥  
11-Q-⑥  
11-N-③  
11-M-③

split into (p100)

As49 MT2 hygro (freeze)

msb-crmA② (Freeze + split) (p100)

11.b.11 (split 1:3) just a bit too heavy

crmA-11.b⑤ split (freeze)

11.b-crmA⑨ split (freeze)

Bring up msb-crmA<sub>2</sub> to FLASK

3 Harvest virus spinner

4 split other spinners

My Spinner:

-  $5.4 \times 10^5$  cells/ml

split 1/2 - freeze cells

-  $4.4 \times 10^5$  / ml / 1 liter removed  
and frozen

EXHIBIT

K1

8 - 100MM

7/13

todo

phone:

Ab in white / red File on Arms desk

Inventory virus bank \* look / + ✓ check

✓ (KBS from freezer) / when brought up

$2 \times 10^5 / \text{ml}$

Develop the gel (Monday)

$7.5 \times 10^7$

✓ (cells frozen + thrown out) ✓

no less than 2x10<sup>5</sup> / ml  
3mls / 200mls cell

(last 200ml / media)

Ad 1 + Ad 6 (Virus stock) (5mls of stock)

Set up 1-100ml → stock media.

grow up 11.6 pre-Absorbed. ✓ Set up \*

293 cells from Kaye (Diplo) OK  
Ginsberg: H5N1 III (earliest stock) (175 flasks)

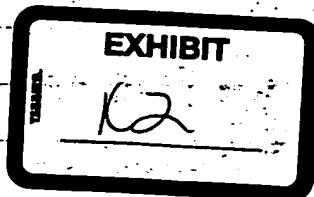
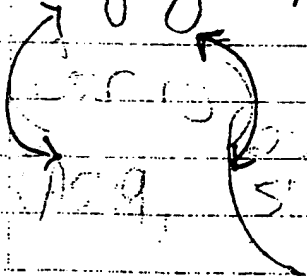
V5103 S-2-1 stock 50ml → 100mm plate → flasks

V5103 S-4-1 spin down / media 2x10<sup>6</sup> FBS 100ml → 100mm plate

(harvested) (960725)

Bayley 11/07/6 (911003) 104-8 loc 50ml → 100mm plate

freeze thaw  
harvested (960725) 104-8



1. spinner 1 liter K.B.'s

Count  $3.4 \times 10^5$  cells/ml

split 500mls into a 3 liter flask add 1.5 liters Joklik's media. Bring original 1 liter spinner up to 1 liter (Joklik's + 10% hrs) serum)

2. 3 liter spinner of K.B.'s infected

Count is  $3.2 \times 10^5$  cells/ml

~~was~~ infect with (Baileys) 11/07/6 911003)

Baileys used was of a new stock grown up in 293 cells. last week Baileys. 96002; (Titer unknown) infected at 11:30 → 12:30

used 200mls of virus into 1 liter of cells for 1 hour. then added back media (2 liters Joklik's + 100 ml denatured Horse serum)

3. develop gels.

4. split 293 give one plate to Ann. (split other cells)

5. look at plaques that are forming.  
Very few new since yesterday  
Count tomorrow.

EXHIBIT

K3



1. Split 293, A549 in 60mm (dishes)
2. Bring 3 liter spinner up to add 1 liter  
Count.  $7.2 \times 10^5$

infect tomorrow w/ (704)

3. New media into all 56-29, 56-25, MSB-2MD, MSB-3MD, CMA-56-③, 11.6-1MD, 11.6 CMA-② + A549-1-Q

4. Harvest infected spinner; Bailey: wash pellet w/ PBS 2x (cold) resuspended it into 24mls total vol of .01 M Tris pH 8.0 mls of cells + tris = 24mls put into snap cap tubes & freeze at  $-70^\circ$ . put in freeze (right inside Lindas lab)

5. Split & freeze ~~200~~ 500mls of KB back up spinner.

Bowlup

6. Cells are not completely infected some are dying so must harvest cells

EXHIBIT

K4

CSC Banded the virus Ad1 960825  
1101/1107 Barleys (960902)

1 freeze (thaw)  
2 sonicate x 2  
3 Vol after son 1101 < 21mls

Ad1 < 19mls

add  $V \times 0.51 = g$  of GCL

10.71g in 1101/1107 Barleys  
9.69g w Ad1

infect 3 liter Spinner with  
dl 717 Vp 891208 Titer  $1.3 \times 10^{11}$

Cell Count 3 liter  $3.5 \times 10^5$   
Vol = 3 liters (3000mls)

$3.5 \times 10^5 \times 3000\text{mls} = 10.5 \times 10^8$  total  
cells

USE 20 pfus / cell

$\frac{1.62 \text{ ul} \times 1.3 \times 10^{11}}{1.3 \times 10^{11}} \times 20 \text{ pfus}$

KB's are healthy no clumping

EXHIBIT

K5

Black (first)

960			9/25	Plaque Assay		
	Uruso	Dil	A	B	C	Comment
1.	1101/1107	$.5 \times 10^{-6}$	9	25	—	(first infection)
		$.5 \times 10^{-7}$	3	1	—	
		$.5 \times 10^{-8}$	Ø	Ø	Ø	
		$.5 \times 10^{-9}$	Ø	Ø	Ø	
		$.5 \times 10^{-10}$	Ø	Ø	Ø	
2	765	$.5 \times 10^{-6}$	TMTCA			
		$.5 \times 10^{-8}$	3	Ø	Ø	
		$.5 \times 10^{-9}$	Ø	Ø	Ø	
		$.5 \times 10^{-10}$	Ø	Ø	Ø	
3.	717	$.5 \times 10^{-8}$	Ø			Very hard to see plaques. today
		$.5 \times 10^{-9}$	Ø	Ø	Ø	
		$.5 \times 10^{-10}$	Ø	Ø	Ø	

EXHIBIT

K6

1101 / 1107

$.5 \times 10^6$   
 $.5 \times 10^7$   
 $.5 \times 10^8$   
 $.5 \times 10^9$   
 $.5 \times 10^{10}$

A

TMTCA  
 42  
 6  
 1  
 0

B

TMTCA  
 53  
 4  
 0  
~~0~~

C

8  
 1  
 0

Comments

small plgs. (flat)

717

$.5 \times 10^6$   
 $.5 \times 10^8$   
 $.5 \times 10^9$   
 $.5 \times 10^{10}$

TMTCA  
 TMTCA ←  
 34  
 1

28  
 3

36  
 3

hard to count  
 because everywhere  
 (very faint)  
 very small

765

$.5 \times 10^6$   
 $.5 \times 10^8$   
 $.5 \times 10^9$   
 $.5 \times 10^{10}$

TMTCA  
 12  
 2  
 0

19  
 1  
 0

1  
 0

EXHIBIT

K7

1101/1107

$1.5 \times 10^6$   
 $1.5 \times 10^{-7}$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

A  
 TMTL  
 9  
 14  
~~8~~  
~~8~~

B  
 TMTL  
 14  
 14  
~~8~~  
~~1~~

C  
 12  
~~8~~  
~~8~~

Comment  
 start off small  
 grow very  
 quickly

717

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTL  
 TMTL  
 59  
 4

45  
 4

55  
 8

Very very  
 small

765-

$1.5 \times 10^{-6}$   
 $1.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $1.5 \times 10^{-10}$

TMTL  
 9  
 2  
 1

11  
 3  
~~8~~

3 monolayer  
 strong

EXHIBIT

18

<div>5</div> <div>1101/1107</div> <div> <math>1.5 \times 10^{-6}</math>  <math>.5 \times 10^{-7}</math>  <math>.5 \times 10^{-8}</math>  <math>.5 \times 10^{-9}</math>  <math>.5 \times 10^{-10}</math> </div>	<div>A</div> <div>           TmTC            all together had            5  <math>\emptyset</math>  <math>\emptyset</math> </div>	<div>B</div> <div>           mold + mTC            to count            7  <math>\emptyset</math>  <math>\emptyset</math> </div>	<div>C</div> <div>           2  <math>\emptyset</math>  <math>\emptyset</math> </div>	<div>Comment</div>
<div>717</div> <div> <math>1.5 \times 10^{-6}</math>  <math>.5 \times 10^{-8}</math>  <math>1.5 \times 10^{-9}</math>  <math>.5 \times 10^{-10}</math> </div>	<div>           TmTC            TmTC            14            5         </div>	<div>           —            TmTC            13            4         </div>	<div>           —            7            10         </div>	<div>small but visible</div>
<div>765</div> <div> <math>1.5 \times 10^{-6}</math>  <math>1.5 \times 10^{-8}</math>  <math>1.5 \times 10^{-9}</math>  <math>1.5 \times 10^{-10}</math> </div>	<div>           4 TmTC            5            1            1         </div>	<div>           3            2            mold         </div>	<div>           3            1         </div>	

1101/1107

$1.5 \times 10^{-6}$   
 $.5 \times 10^{-7}$   
 $.5 \times 10^{-8}$   
 $.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

A

TmTC  
 TmTC  
 no new aed  
 Ø  
 Ø  
 Ø

B

TmTC  
 Ø  
 Ø  
 Ø

C

TmTC  
 Ø  
 Ø

Comment

717

$1.5 \times 10^{-6}$   
 $.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

TmTC  
 TmTC  
 TmTC  
 3

TmTC  
 TmTC  
 Ø

TmTC  
 2

765

$1.5 \times 10^{-6}$   
 $.5 \times 10^{-8}$   
 $1.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

TmTC  
 Ø  
 Ø  
 Ø

2  
 Ø  
 throw out

monolayer  
 gone  
 Ø  
 Ø

EXHIBIT

K10

1101/1107

$1.5 \times 10^6$   
 $1.5 \times 10^{-7}$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

A	B	C	Comment
---	---	---	---------

TMTA  
 7  
 3  
 0  
 0

2  
 5  
 0  
 0

6  
 0  
 0

plg came  
 up quickly  
 when taped  
 off

717

$1.5 \times 10^6$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

TMTA  
 TMTA  
 16  
 3

TMTA  
 17  
 4

19  
 7

765

$1.5 \times 10^6$   
 $1.5 \times 10^8$   
 $1.5 \times 10^9$   
 $1.5 \times 10^{10}$

TMTA  
 5  
 1  
 0

3  
 0  
 0

0  
 0

EXHIBIT

K11



1101/1107

A

B

C

Comment

 $1.5 \times 10^6$ 

TMTC

TMTC

-

 $1.5 \times 10^{-7}$ 

TMTC

TMTC

-

 $1.5 \times 10^8$ 

+1

+2

+0

 $1.5 \times 10^9$ 

+0

0

+0

 $1.5 \times 10^{10}$ 

0

~~X~~

0

717

 $1.5 \times 10^{-6}$ 

TMTC

-

-

 $1.5 \times 10^{-8}$ 

TMTC

-

-

 $1.5 \times 10^{-9}$ 

TMTC

-

-

 $1.5 \times 10^{-10}$ 

+0

+2

+0

765

 $1.5 \times 10^{-6}$ 

TMTC

-

-

 $1.5 \times 10^{-8}$ 

+1

+1

-

 $1.5 \times 10^{-9}$ 

+0

+0

+0

 $1.5 \times 10^{-10}$ 

+1

+0

-

EXHIBIT

K12

	A	B	C	Comment
1101/1107				
$1.5 \times 10^6$	mold	34		
$1.5 \times 10^{-7}$	thru	blended together thru		
$1.5 \times 10^8$	5	4	9	Really big
$1.5 \times 10^9$	$\emptyset$	$\emptyset$	$\emptyset$	
$1.5 \times 10^{10}$	$\emptyset$	$\emptyset$	$\emptyset$	
765				
$1.5 \times 10^6$	TMT			
$1.5 \times 10^8$	TMT			
$1.5 \times 10^9$	4	3	12	monolayer looks bad
$1.5 \times 10^{10}$	$\emptyset$	$\emptyset$	$\emptyset$	no new just bigger
768				
$1.5 \times 10^6$	TMT			
$1.5 \times 10^8$	TMT			
$1.5 \times 10^9$	TMT			
$1.5 \times 10^{10}$	3	$\emptyset$	2	

EXHIBIT

113

		A	B	C	comment
760	$1.5 \times 10^{-8}$ $1.5 \times 10^{-9}$ $1.5 \times 10^{-10}$	TMTZ 37 3	TMTZ 29 1	TMTZ 26 4	
327	$1.5 \times 10^{-8}$ $1.5 \times 10^{-9}$ $1.5 \times 10^{-10}$	TMTZ 60 18	TMTZ 74 17	74	
1101/1107	$1.5 \times 10^{-6}$ $1.5 \times 10^{-7}$ $1.5 \times 10^{-8}$ $1.5 \times 10^{-9}$ $1.5 \times 10^{-10}$	TMTZ TMTZ TMTZ 66 8	TMTZ TMTZ TMTZ 64 7	70	Big Alp
702	$1.5 \times 10^{-8}$ $1.5 \times 10^{-9}$ $1.5 \times 10^{-10}$	TMTZ TMTZ 36	TMTZ TMTZ 30	TMTZ 36	

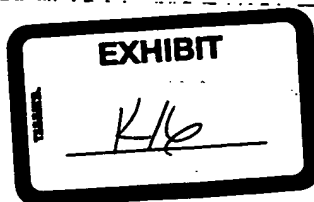


		A	B	C	comment
760	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	TMT 44 5	TMT 40 4	TMT 42 6	
327	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	TMT 64 18 <i>really TMT</i>	TMT 74 16	72 37 <i>Missed counting first time</i>	
1101/1107	$.5 \times 10^{-6}$ $.5 \times 10^{-7}$ $.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	TMT TMT TMT 25 11	<hr/> 21 9	22	<i>med rice plg.</i>
702	$.5 \times 10^{-8}$ $.5 \times 10^{-9}$ $.5 \times 10^{-10}$	TMT <del>21</del> <i>almost 21</i> 15	TMT 24 14	22 15	

EXHIBIT

KIS

		A	B	C	common
760	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMTc 10 1	TMTc 20 3	TMTc 15 1	small
327	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMTc TMTc 10	TMTc TMTc 8	TMTc TMTc 9	
1101/1107	$5 \times 10^{-6}$ $5 \times 10^{-7}$ $5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMTc TMTc TMTc 4 0	— TMTc TMTc 5 0	4 —	
702	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMTc TMTc 3	TMTc 3	5	



2m/miles

760

$.5 \times 10^{-8}$   
 $.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

A  
 TMTCA  
 TMTCA  
 4

B  
 TMTCA  
 TMTCA  
 3

C  
 3  
 1  
 common  
 very small easy to count.

327

$.5 \times 10^{-8}$   
 $.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

TMTCA  
 TMTCA  
 3

TMTCA  
 TMTCA  
 5

—  
 TMTCA  
 6

1101/1107

$.5 \times 10^{-6}$   
 $.5 \times 10^{-7}$   
 $.5 \times 10^{-8}$   
 $.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

TMTCA  
 TMTCA  
 TMTCA  
 0  
 2

—  
 TMTCA  
 TMTCA  
 1  
 1

0  
 0  
 very acidic

702

$.5 \times 10^{-8}$   
 $.5 \times 10^{-9}$   
 $.5 \times 10^{-10}$

TMTCA  
 TMTCA  
 1

TMTCA  
 0

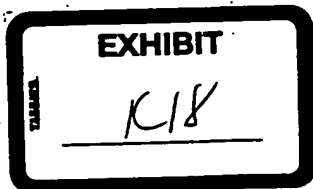
TMTCA  
 1  
 very close hard to count

EXHIBIT

147

		A	B	C	common
760	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMCA TMCA 3	TMCA TMCA 2	2	
327	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMCA TMCA 8	TMCA TMCA 5	TMCA 2	
1101/1107	$5 \times 10^{-6}$ $5 \times 10^{-7}$ $5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	$\emptyset$	$\emptyset$	$\emptyset$	all plates too acid no new colonies
702	$5 \times 10^{-8}$ $5 \times 10^{-9}$ $5 \times 10^{-10}$	TMCA (throw out) TMCA $\emptyset$	TMCA $\emptyset$	TMCA $\emptyset$	throw out

Keep plates  $5 \times 10^{-10}$  760, 327  
 still growing pla



		Plaque Assays (% of final plaque #)							
Virus		5d	7d	12d	13d	16d	19d	25d	30d
①	1101/1107	0.	18.3	55.	67.9	80.7	80.7	83.5	100.9.
②	sub 765	2.6	32.8	57.8	65.5	79.3	81.	83.6	100.
③	dl 717	0.	10.3	33.8	54.4	82.4	89.7	92.6	100.

titer of 1101/1107 :  $6.61 \times 10^9$  /ml  
(harvested from KB cells at day 2)

		10-15-96 Plaque Assays (% of final plaque #)				
Virus		8d	10d	13d	16d	21d
①	pm 760	32.4	78.	94.2	97.7	100.9.
②	dl 327	32.1	65.4	82.1	90.7	100.
③	dl 1101/1107	65.7	94.6	98.7	100.	100.
④	dl 702	64.2	91.8	98.7	100.	100.

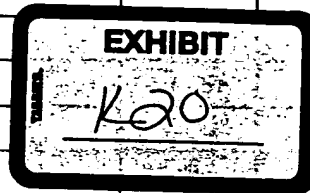
titer of 1101/1107 :  $1.95 \times 10^{11}$  pfu/ml  
(harvested from KB cells at day 3)

EXHIBIT

K19



	VIRUS	From	INF	Harvest	spin	plag	Comments	Freezer/b
1.	Ad4Δ19K (V558)	961016	961018					
2.	Ad4ΔE <sub>3</sub> (96103)	961104	961106					
3.	Ad4ΔE <sub>3</sub> B (V556)	961031	961102					
4.	1101/1107 (Baileys)	960905	960907	✓	✓			1-1
5.	1101/1107 2nd batch	960917	960919	✓	✓			1-1
6.	d1702 (V533)	961002	V2day	✓	✓			1-2
7.	d1773 (V5230)	960909	V2day	✓				101-5
8.	d1717 (V5118)	960906	960908	✓	✓			2-2
9.	327 (V5212)	960914	960917	✓	✓			5-5
10.	Ad1760 (V5227)	960915	960917	✓	✓			4-6
11.	Ad5 (V5198)	960907	960908	✓	✓	Great bands		101-2
12.	Ad1 (sup)	960905	960907	✓	✓			2-1
13.	d1762 (Repeat)	960908	960908	✓	✓	Bad No plagues		2-3
14.	Ad11 Sup	961030	961002					
15.	dL797 (V5243)	960922	960924					
16.	Ad3					first spin		
17.	Ad6 SUP	960801	960803	✓	✓	Good bands		101-2
18.	Ad9 (sup)	970209						
19.	dL703 V550	961001	961003	✓				
20.	dL704 (V5167)	960802	960804	✓	✓	thin bands		5-2
21.	dL748 (V5181)	960708	960810	✓	✓			13-6
22.	dL798 (V5188)	960708	960710	✓	✓	Good		13-4
23.	dL763 (V5214)	960703	960705	✓	✓	Bad Bands look good		2-3
24.	dL765 (V5225)	960824	960826	✓	✓			101-8
25.	dL739 (V5223)	961023	just plg	✓	✓			
26.	dL754 (V5243)	960202	just plg	✓	✓			
27.	dL712 ?	950503	—	✓	✓	Plays good		
28.	7001 ?	960724	960726	✓	✓			
29.	dL250 sup	950616	950618	✓	✓	Big plagues / nice bands		
30.	Ad41 sup/293	(293)	→					
31.	Ad40 sup/293	(293)	→					
32.	Ad707 (V5112)	961008	961110					
33.	Ad740 V5240	960612						
34.	dL768 (Apr 29)	961120	961122					
35.	Ad1KS (V5224)	970305		✓				
36.	Ad11	970205	970205	✓	✓	Great bands		
37.	Ad11	970215	970215	✓	✓	Great " !		



Chris 4/62

Low affinity Nerve growth receptor = .1 ug / ul

Viruses from Kostaya: grow in KB's first AB49  
then KB.

= Viruses from Bowling - grow in AB49 first then  
then in KB! 2 flasks

Set up 2 flasks for bowling virus. pmt 116  
AAA

= KB's ready to infect in 3 liter spinner  
counts

1 liter bring up to 1300 liters split  
tomorrow.

dl 707:  $1.57 \times 10^{10}$

count  $3.8 \times 10^5 \times 9.9 \times 10^8$  cells / liter

$$\frac{0.99 \times 10^8}{1.57 \times 10^{10}} \times 10 \text{ PFU's} = 63 \text{ u}$$
$$\frac{0.99 \times 10^8}{1.57 \times 10^{10}} \times 5 \text{ PFU's} = 315$$

Infect.

EXHIBIT

K21

4/28/mondas

1. set up AS49 for virus from Costag
2. split MCF-7 for transfection of  
Nerve growth factor.
3. change media on MCF-7 - transfections  
11.6 - MCF-7 ~ 5-6 clones per dish  
MS6 - MCF-7 ~ 3-4 " " "  
except for one dish which has alot more

thus:

set up transfection w/  
Gow Affinity Nerve growth factor

= 3 dishes  
+ 11.6 = 3 dishes  
2 controls

use new prep.

EXHIBIT

K22

Coverslips of mcf-7.

is for affinity Nerve growth factor .2 ug/ul

use solution

$A = (PK \log FR MN) \cdot 2 \text{ ug/ul} = 25 \text{ ul/dish}$

NGF ONLY

3 ref only

A = 75ul DNA + 800ul opti DMEM =

B = 36ul lip + 1300ul opti DMEM

A+B for 30min 2.4 ml opti

NGF + 11.6

A 5.7 11.6 + 75ul + 300ul opti DMEM

B 36ul lip + 300ul opti DMEM

A+B for 30min 2.4 ml opti

2 dishes not transfected = for control

- mix A+B let sit R.T for 30min, Add Raman-nosin
- 1ml per dish / for five hours
- then add 20% media (1ml) let incubate O/N
- stain tomorrow.

1 dish

pMT2 2.2 + 100 opti DMEM

12 lipo + 100 opti DMEM

Add 1ml 20% on transfection / Add 2% to infection

Set up flasks 293 (110 virus)

Set up 4 A549 plates (100ul to be absorb ab)

Add Costas Virus to flasks - put split K B S

EXHIBIT

1623

5/2 Friday

- FREEZE FAS cells - pool
- V infection
- split all cells /
- split KB's back

5/6

- split FAS to two flasks - for Koshy
- clones picked
- infect the other ~~spinner~~

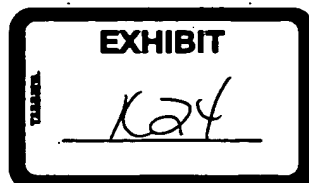
$$4.5 \times 10^5 \text{ cells/ml} / \times 3000 \text{ nls} =$$

13,500 cells.

cells look ok: added 5mls of  
the virus 45.5 from Koshy to  
the spinner

(tomorrow pick clones.)  
get cells from Mohan  
make Jakl's media / ok

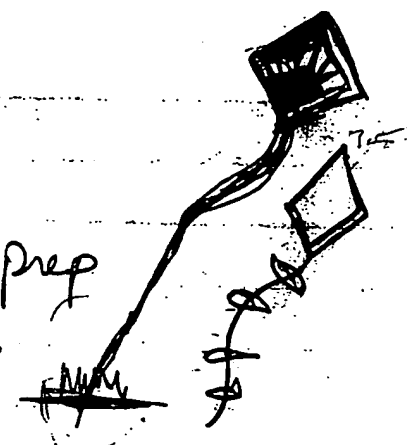
*EW*



1:30

5/B

797?



freeze + thaw + spn to CS prep  
viruses

20 ml  
19 ml  
20 ml  
19 ml

KD-1	1101/1107	ADP	970508
PME	11.6	AAA	970503
dl	707		970423
dl	751		970324

Viruses in warm room X2 thaw.

~~vitamin C~~ also

20 ml

dl 707 970315

7 1/2  
147

split ASTC cells to Flasks for 797 + others  
1. total -

IN Freezer, need Sonicated in beater

location

PME = 1, 7  
KD = 9, 3  
751 = 11, 5  
707 (970315) 8, 2  
707 = 6, 12

set machine on  
4° / 35K for  
O/N ↔

EXHIBIT  
K25

5/14

Viruses taken out of Centrifuge

(970508) <sup>(45-47)</sup> KD-1 (Kostya) 1101/1107 ADP: <sup>harvest</sup> ~ 4 mls (small thin bands)  
 (970324) dl 751 (nice thick bands) good = ~ 4 mls  
 (970315) dl 707 (very thick big bands great) = ~ 9 mls  
 (970423) dl 707 (nice bands) good = ~ 6 mls

(970503) PME-11.6 AAA (Very nice bands) good ~ 5 mls/3  
 (BLY ADP-AAA)

(Note) Don't dilute KD-1 very much got alot of extra liquid w/ viruses band ~ 2 mls of actual virus.

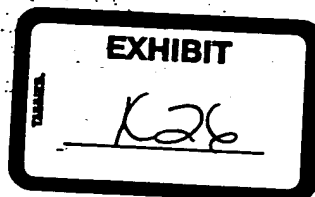
Dilute with TSB ~ 5x-10x

	tubes	LOC
KD-1 dilute bring up to (10 mls)	5	4-4
dl 751 bring up to (16 mls)	8	2-5
dl 707 (970315) bring up to (24 mls)	12	2-4
dl 707 (970423) bring up to (16 mls)	8	4-3
PME-11.6 AAA = bring up to (14 mls)	7	4-4
BLY (ADP-AAA)		

(plaque assay viruses) 751 original 53-3/60-B  
 one tube of each in my box to plaque 707 original

need to infect 3 tubes w/ H5 dl 111  
 H5 dl 110  
 dl 205

from flasks AS49.



5/20/97

Agro assay. (Titer virus)

dl 751 (970324)

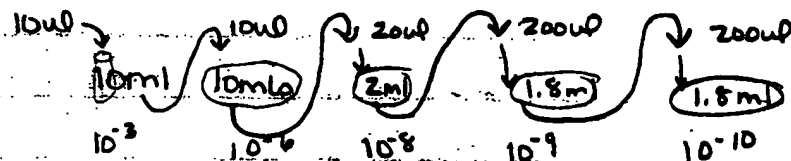
dl 707 (870315)

dl 707 (970423)

DME - 11.6 ATP (BLY-ADP-AAA) (970503)

KD-1 (970508) (Kostya)

dilute all viruses 10uL to 10mls ( $10^{-3}$  dilution)



45  
605

2 -  $10^{-8}$   
 3 -  $10^{-9}$   
 3 -  $10^{-10}$

\* each

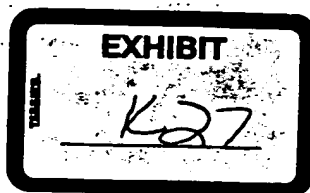
50  
5  
2  
1  
43

100 mls 2x DME  
 10 mls Sodium Bi  
 4 mls FBS  
 2 mls Glut  
 86 mls AGAR

Add virus to the 3liter spinner - dl 110 virus

3 liter Count:  $4.2 \times 10^5$  cells/ml. use 1ml cells.  
 20110

1 liter Count:  $(2.7 \times 10^5 \text{ cells/ml})$  split.





6/2

stand 5/23

	A	B	C
$5 \times 10^{-8}$	TMTL	TMTL	/
$5 \times 10^{-9}$	10	bact	10
$5 \times 10^{-10}$	2	✓	1

A	B	C
TMTL	TMTL	/
9	3	10
3	✓	✓

707  
(5/10)  
WAGS

$5 \times 10^{-8}$	14	15	/
$5 \times 10^{-9}$	2	5	2
$5 \times 10^{-10}$	✓	✓	✓

68	47	/
8	2	✓
✓	✓	✓

707

$5 \times 10^{-8}$	13	15	/
$5 \times 10^{-9}$	3	✓	✓
$5 \times 10^{-10}$	✓	✓	3

30	17	/
2	3	✓
✓	✓	✓

KD-1

$5 \times 10^{-8}$	Total 162	163	Total 162
$5 \times 10^{-9}$	Total 46	49	Total 46
$5 \times 10^{-10}$	1	✓	✓

TMTL	TMTL	/
3	1	1
1	✓	2
	$8.1 \times 10^9$	

PMF  
(nice size  
pieces)

$5 \times 10^{-8}$	74	78	/
$5 \times 10^{-9}$	16	11	28
$5 \times 10^{-10}$	4	3	2

TMTL	TMTL	/
12	14	7
1	✓	2

V1 38 injections with 1101/1107 and d1308

11/24

MOI      dividing      quiescent      5.43  
 2.1  
 1  
 10  
 100

16 35 mm V1 38  
 8 35 mm A549  $7.5 \times 10^5$  all/dish

injections 11/25  
 100  $7.5 \times 10^5$  cells

$5 \times 10^4$	0.1	MOI
$5 \times 10^5$	1	
$5 \times 10^6$	10	
$5 \times 10^7$	100	

$1.34 \times 10^4$   
 101/107 (96737)  
 $3.8 \times 10^5$

$3.1 \times 10^2$   
 10x61 6.1

~~7.110<sup>3</sup>~~  
~~312~~  
~~10x61~~  
~~6.1~~

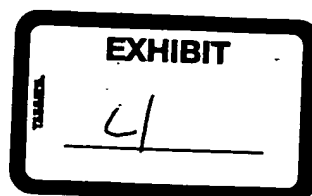
change medium on V138 to 0.2% FCS

11/22 infect started V138 with

d303 (950511)  
 100  
 10 MOI  
 1  
 0.1

1101/1107 (910917)  
 100  
 10 MOI  
 1  
 0.1

BEST AVAILABLE COPY



# ADP Western

infect AS41 with 303, KDI 12/9

$10^6$  cells / dish  $8.09 \times 10^6$  / ml 303  $\Rightarrow 8.09 \times 10^6$  / ml  
 $50 \mu\text{m}/\text{cell} \Rightarrow 6.18 \mu\text{l}$

$6.12 \times 10^6$  / ml KDI  $\Rightarrow 6.12 \times 10^6$  / ml

$50 \mu\text{m}/\text{cell} \Rightarrow 8.16 \mu\text{l}$

Harvest 33 h p.i.

$\phi$	0.100		
mark	0.100	100	5 $\mu\text{l}$
303	0.059	173	2.8 $\mu\text{l}$
KDI	0.060	140	3.6 $\mu\text{l}$

4

27  
26  
45  
31  
21.5  
14.4  
6.5

10 small 15% SDS OAC

[mark, 303, KDI] 3X, BR

housek / mark 12.5 10%, 456/5%

black 10% milk ON

cut 1st ab

4 12532II  
 5 12531  
 6 100578

1:400

Wash

2nd ab

3X } a rabbit HRP

Wash

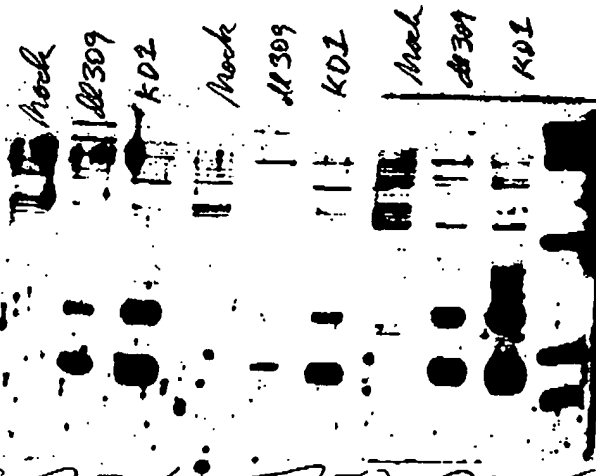
5X

EXHIBIT

L2



- KD1 expressed Ad5 Ab well  
 - Can detect Ad5 by Western  
 - Prelim - looks like KD1 expresses more ADP than 309?  
 Used 500 PFA 7 KD1  
 Used 50 PFA 7 KD1



ADP -  
 ADP -

3 diff antibodies

12532E 12531 100578  
 100 dilution Use this

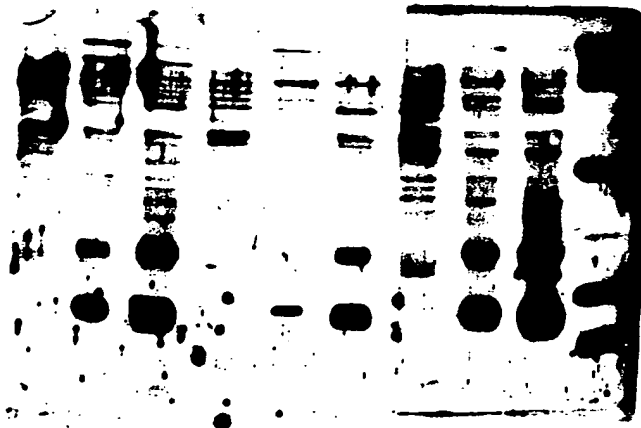
2ADP Western  
 3d 1 pe

Not sept - Time course  
 KD1  
 KD2  
 KD3  
 1101/1107

@



12/12/87



WDI  
308  
mch

WDI  
308

mch

WDI  
308  
mch

100574

1252

12521



EXHIBIT

tabb

64